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Synthesis and characterization of dextran esters as coating or matrix systems for oral delivery of drugs targeted to the colon

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Abstract:

Different dextran esters with various degrees of substitution (1, 2 and 3) were synthesized by esterification reaction, with three acid anhydrides: acetic anhydride, propionic anhydride, and butyric anhydride, separately. These modified polysaccharides were characterized by FT-IR, ^{1}H NMR and ^{13}C NMR spectroscopies. Enzymatic degradation of biopolymers by dextranase was also studied. The polymers showing the best degradation profiles were chosen to design blended free films in combination with a polymethacrylate (Eudragit® RS 30D) as a sustained release system for targeting to the colon. These free films were evaluated by permeability of theophylline used as tracer in different *in vitro* media of the gastro intestinal tract, in presence or in absence of dextranase. From these studies, it was concluded that dextran esters having the lower degree of substitution and constituted of short carbohydrate chains showed the best and significant enzymatic degradation and could be used as a promising carrier for specific colon drug delivery system.

Keywords: Colon-Specific Drug Delivery; Polysaccharides; Dextran; Dextranase; Dextran esters; Enzymatic Degradation; Eudragit® RS 30D; Sid-by-side diffusion cell

Introduction

The oral route is the most common and convenient of the existing administration methods for systemic drug delivery. It affords high patient acceptability, compliance, and ease of administration. Moreover, the cost of oral therapy is generally much lower than that of parenteral therapy [1,2]. Research interest in the area of colonic drug delivery has been fuelled by the need to better treat pathologies of the colon that range in seriousness from constipation and diarrhoea to debilitating inflammatory bowel diseases, ulcerative colitis and Crohn's disease, through to colon carcinoma. Targeted oral drug delivery to the colon would therefore ensure a direct treatment at the disease site, lower dosing and a reduction of systemic side effects. Aside from local treatment, the colon can also be utilised as a portal for the entry of drugs into the bloodstream for the purpose of systemic therapy. For example, molecules that are degraded and/or poorly absorbed in the upper gut, such as peptides and proteins, may be better absorbed from the more benign

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environment of the colon. In addition, systemic absorption from the colon can also be used as a means of achieving chronotherapy for diseases that are sensitive to circadian rhythms such as asthma, angina, and arthritis [3,4].

Amongst the different strategies to reach the colon described in the literature [5], the simplest and less costly strategy consists using polyacrylate derivatives or polysaccharide-polyacrylate derivatives such as ammonio methacrylate copolymer. They can be a good choice for people suffering of colon disease for which any variability of pH into the colon occurs. Besides these synthetic polymers, the use of polysaccharides degradable by enzymes and/or bacteria present into the colon can also be a good strategy. Furthermore, polysaccharides are found in abundance, have wide availability, are inexpensive and available in a variety of structures with a variety of properties. They can be easily modified chemically and biochemically and are highly stable, safe, nontoxic, hydrophilic and gel forming and in addition biodegradable, which suggests their use in targeted drug delivery systems [6]. Furthermore, many of the polysaccharide-based delivery systems shield the drug from the hostile environments of the upper GIT [7,8].

Dextrans are a class of polysaccharides consisting of α-1, 3 and α-1, 6 glycosidic linkages and having unique properties, such as biodegradability at specific body sites, e.g. the colon. Dextranases are the enzymes which hydrolyse these glycosidic linkages. Dextranase activities on the colon are shown by anaerobic gram-negative intestinal bacteria especially the Bacteroides. The Bacteroides are the numerically predominant anaerobes in the colonic region of humans. They number about 10^{11} per gram of intestinal contents and constitute approximately 30% of total cultivable gut flora. The majority of strictly anaerobic bacteria in the colon are saccharolytic. The bacteria drive their energy from the fermentation of carbohydrates, which results in the production of short chain fatty acids [9]. Dextranase activity in human caecostomy effluent samples was reported to be 650 DU/ml, equivalent to 30 U/ml measured in conventional enzyme units, and 15 DU/g equivalent to 0.69 U/g in human fecal samples [10]. Due to its degradability in the colon, dextran is an ideal candidate for oral drug delivery systems. However, dextran itself cannot be used as drug carrier due to its high water solubility and therefore the first need is to make it more hydrophobic [11].

The aims of this study were to synthesize and characterize new dextran esters such as dextran acetate (Dex.Ace), dextran propionate (Dex.Pro) and dextran butyrate (Dex.But) with three degrees of substitution by esterification reaction, to be used as coatings or as a matrix systems for solid oral drug dosage forms (tablets, capsules and mini granules) in both cases as carriers for site specific drug delivery to the colon. Preparation of biodegradable matrix films consisted of dextran esters, as biodegradable modified polysaccharides into the colon, and Eudragit RL 30 D as a sustained-release polymer [12]. The permeability of those free films mixtures into different media mimicking the gastrointestinal tract was also realized.

Materials and methods

Materials

Dextran (Dex) of MW 67500 was purchased from Pharmacosmos (Holbaek, Danemark). Dextran was dried in a vacuum oven for 24 h at 50 °C before use. All chemicals were used without further purification. Acetic anhydride, butyric anhydride, propionic anhydride, dextranase (EC 3.2.l.l1) from Penicillium, ammonium acetate, sodium azide, sodium potassium tartrate, phenol, 3,5-dinitrosalicylic acid, sodium sulfite, dimethyl sulfoxide (DMSO), ethyl acetate, chloroform, ethanol, methanol, tetrahydrofuran, acetone, 2-propanol, dichloromethane, 4-(dimethylamino) pyridine (DMAP) and triethyl citrate were purchased from Sigma-Aldrich Chemical Company (Saint Quentin Fallavier, France). Theophylline monohydrate was obtained from Cooper Rhone (Melun, France). Eudragit® RS 30D was obtained from SPCI (St-Denis La Plaine, France).

Synthesis of different dextran esters

The general synthesis of dextran esters is shown in Figure.1. 10 g of dry dextran and 4-Dimethylamino pyridine (DMAP) 10% w/w were dissolved in dimethyl sulfoxide (DMSO), in a 500 ml two-neck round-bottomed flask equipped with a stirrer, a condenser and a drying tube, and heated to 80 °C. The mixture was stirred until it was completely dissolved. Then, the required amount of anhydrides acid are slowly added drop by drop, and the mixture was stirred for a determined period as shown in Table 1, then cooled down at room temperature. The obtained dextran esters were precipitated in 1000 ml of distilled water and filtrated. Then, the precipitate was dissolved into 300

ml ethyl acetate, washed three times with distilled water, two times with a saturated solution of sodium bicarbonate, two times with a saturated solution of sodium chloride, and finally two times with distilled water, respectively. Then, after evaporation of the solvent, the residue was dissolved in a small amount of methanol. Finally, the product was isolated by precipitation into 1000 ml of distilled water and filtrated. The precipitated polymer was dried at 50 C° under vacuum during 24 hours.

Table 1: The degree of substitution (DS), the esterification conditions, and the yields of dextran esters

Dextran esters	DS^{th}	DS^{Cal}	Volume of anhydride acid (ml)	Volume of DMSO (ml)	Time of reaction (h)	Yield (%)
	3	3.02	30.2	80	4	94.43
Dextran butyrate	2	1.98	20.1	80	4	92.16
	1	0.98	10.07	80	4	90.12
	3	2.97	24.1	80	4	93.54
Dextran propionate	2	1.92	16.1	80	4	92.23
	1	0.97	8.03	80	4	90.01
	3	2.93	17.9	200(1)	12(2)	92.13
Dextran acetate	2	1.91	11.9	200(1)	12(2)	91.83
	1	0.96	5.97	200(1)	12(2)	55.05

Characterization and solubility

The chemical structure of dextran and the different dextran esters were all characterized by FT-IR (Nicolet 380- Thermo electron, USA), and ^{1}H NMR, ^{13}C NMR (Burker Avance PDX300 MHz spectrometer, Karlsruhe, Germany). For FT-IR characterization all samples were dried into a vacuum oven for at least 24 h before analysis. For ^{1}H NMR and ^{13}C NMR the samples were dissolved in deuterated DMSO-d_6 or $CDCl_3$ (both were obtained from Euriso-top, Gif-sur-Yvette, France). Chemical shifts (δ) are reported in parts per million. For central lines of internal references, DMSO-d_6 (δ = 2.50) and $CDCl_3$ (δ = 7.24) were used. The degree of substitution (DS) was estimated from the ^{1}H NMR spectras [13,14].

The solubilities of the different dextran esters were determined by immersing 20 mg of each sample into a solvent for 24 h [15] followed by a filtration and a drying of the non soluble fraction.

Enzymatic degradation of different Dextran esters

30 mg of different dextran esters were incubated in 30 ml of ammonium acetate buffer (5 mM, pH 5.5) containing 0.02% (w/v) of sodium azide, with three concentrations of dextranase (0.5, 5 and 10 U/ml) respectively, at 37 °C. The enzyme solution was prepared just before use. Periodically, samples of 2 ml were taken at different times (2, 6, 24 and 48 h), and heated at 95 °C for 10 min to inactivate the enzyme.

The concentration of reducing oligosaccharides was determined as described by Franssen et al. [16,17]. Typically, 200 µl of sodium sulfite solution (30 mg/ml) and 3 ml of Sumner reagent (0.2 g/ml of sodium potassium tartrate, 10 mg/ml of dinitrosalicylic acid, 10 mg/ml of sodium hydroxide and 2 mg/ml of phenol) were added to a sample of 2 ml and were incubated for 15 min at 95 °C. After cooling to room temperature, the absorbance was measured at 620 nm and the concentration of reducing oligosaccharides was calculated using glucose solutions as references. Each experiment was performed in triplicate and the results were in agreement within ±3% standard error.

Preparation of free films

A 10% (w/v) solution of Eudragit® RS 30 D (ERL) was prepared in distilled water, and then a fixed amount of triethyl citrate (20% to the total polymer

content) was added as plasticizer. Required amount of dextran acetate (DS1) powder was dissolved in distilled water. Dextran acetate solution was gently added to Eudragit® RS 30 D solution with continuous stirring. The ratios of dextran acetate to Eudragit® RS 30 D content were 0, 10, 20 and 30% (w/w) [18]. Identical volumes from the resulted suspension were transferred to Teflon plates and dried into a ventilated hood at temperature ambient for 48 h for complete drying. The integrity of the films was checked visually and the film thickness was measured at five different places by using a micrometer (Käfer, Germany). Free films were stored in a desiccator with 50% RH obtained by a saturated solution of magnesium nitrate hexahydrate at room temperature until use.

Permeability test

Isolated films of the polymers were mounted between the donor and the acceptor compartments of a side-by-side diffusion cell (Grown Glass Co., Inc., Somerville, New Jersey, USA) having a diffusion area of 3.46 cm^2. The temperature of the cells was kept at 37 ± 0.5 ºC throughout the experiments by continuous circulating of water and each compartment was stirred continuously with a magnetic stirrer. Different experimental conditions were set up to examine the permeability of drugs through the polymer films. For theophylline, used as a hydrophilic model drug, the initial concentration of these one in the donor compartment was 3.0 mg/ml. To achieve the sink conditions for permeability experiments, nearly saturated concentration of theophylline was poured into the donor compartment. The donor and acceptor compartments were both composed of simulated gastric fluid (SGF) without pepsin, simulated intestinal fluid (SIF) without pancreatin with pH 6.8 (USP XXVIII, 2005) and simulated colonic fluid (SCF, 0.05 M phosphate buffer, pH 6.4) added with 0.5 U/ml of dextranase respectively. All of the permeability experiments were carried out for 3 h. At predetermined time intervals, samples of 10 ml were taken from the receptor cells and replaced with fresh medium. The contents of the acceptor cells were assayed spectrophotometrically for theophylline at 272 nm. Each permeation experiment was repeated three times and the cumulative amount of drug permeated and corrected for the acceptor sample replacement was plotted against time.

Applying Fick's first law of diffusion in sink conditions, the permeation rate of drug was defined as:

$$\frac{d_M}{d_t} = \frac{DSKC_d}{h} = PSC_d \quad (1)$$

Where M, was the amount of drug diffused (mg) at time t; D, the diffusion coefficient; S, the effective diffusion area (cm^2); C_d, the concentration of drug in the donor cell; h, the thickness of membrane; and P, was the permeability (cm/s). Then, the permeability was obtained by the following equation:

$$P = \frac{Slope}{SC_d} \quad (2)$$

Where the slope was obtained from the plot of the amount of drug permeated versus time [19].

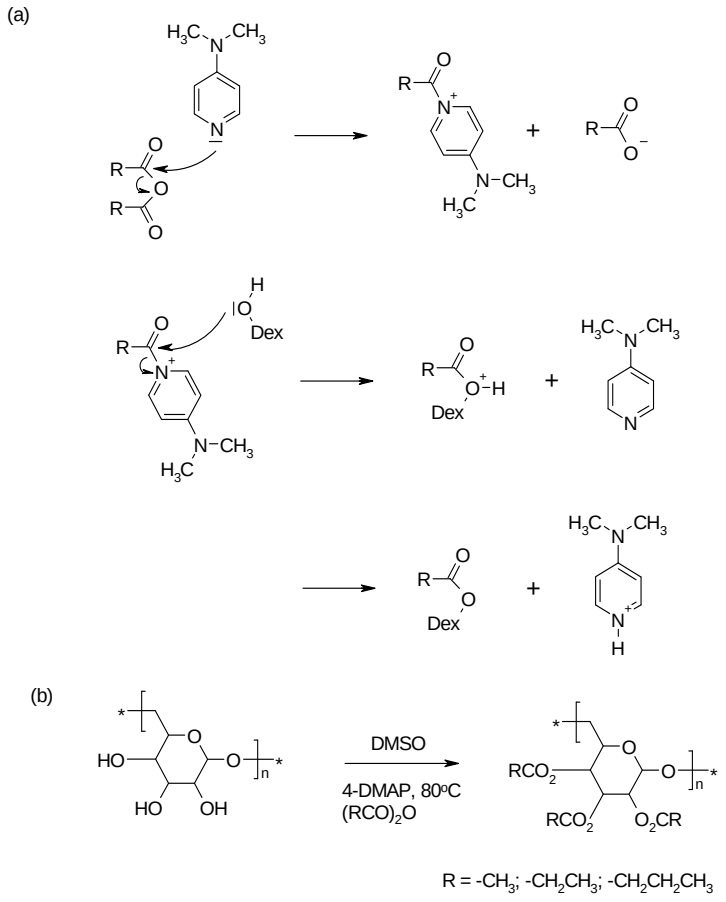
Results and discussion

Synthesis and characterization of different dextran esters

A series of different dextran esters, acetate, propionate and butyrate, with various degrees of substitution (DS: 1.0, 2.0 and 3.0) were synthesized by esterification under anhydrous conditions (Fang et al., 1999).

Figure 1 gives the general scheme of the esterification reaction, and the mechanism of reaction [20]. Dextran reacted with acid anhydride in DMSO in presence of 4-DMAP used as catalyst at 80 °C [21-23]. All or some of the hydroxyl groups of dextran were replaced by carboxylic groups to give dextran esters (acetate, propionate and butyrate).

The structure of the different dextran esters were confirmed by FT-IR, ^1H NMR and ^{13}C NMR spectroscopies. Figure 2 shows the FTIR spectras of the dextran and the different dextran esters. By comparing the different FTIR spectras of dextran butyrate, we can show a decrease in the O-H band (3340 cm^{-1}) and an increase in the three major ester bands [the C=O band (1740 cm^{-1}), the C-O band (1250 cm^{-1}), and the C-CH_3 band (1350 cm^{-1})], providing evidence of esterification as reported by Sun et al. [24]. Same results were observed for dextran propionate and dextran acetate.



(R= CH_3 dextran acetate, CH_2CH_3 dextran propionate and $CH_2CH_2CH_3$ dextran butyrate)

Figure 1. General scheme of the synthesis dextran esters (a) Mechanism of esterification reaction (b) General reaction.

The chemical structures of the different dextran esters were also examined by ^{1}H NMR spectroscopy. The characteristic resonance peaks corresponding to the protons in methylene groups and other five methanol groups of dextran can be observed at 3.4- 4 ppm and 4.9 ppm. In addition, the signals of protons in dextran butyrate appear in the spectrum at 0.92 ppm (-CH_2-CH_2-C$\underline{H}_3$), 1.6 ppm (-CH_2-C$\underline{H}_2$-CH_3), and 2.3 ppm (-C$\underline{H}_2$-CH_2-CH_3), the signals of protons in dextran propionate appear at 1.08 ppm (-CH_2-_C$\underline{H}_3$), and 2.23 ppm (-C$\underline{H}_2$- CH_3) and the signals of protons in dextran acetate appear at 1.9 ppm (-C$\underline{H}_3$). For the ^{13}C NMR spectra, the resonance peaks of the six carbons in dextran can be observed at 65.6- 97.7 ppm, the resonance peaks corresponding to dextran butyrate appear at 13.5 ppm (-CH_2-CH_2-C$\underline{H}_3$), 18.8 ppm (-CH_2-C$\underline{H}_2$-CH_3), 35.8 ppm (-C$\underline{H}_2$-CH_2-CH_3) and 172.2 ppm (carbonyl croups C=O), for dextran propionate appear at 9.0 ppm (-CH_2-C$\underline{H}_3$), 27.3 (-C$\underline{H}_2$-CH_3) and 173.3 ppm (carbonyl croups C=O), and for dextran acetate appear at 20.9 ppm (-C$\underline{H}_3$) and169.8 ppm (carbonyl croups C=O) as reported by Wang et al. [25].

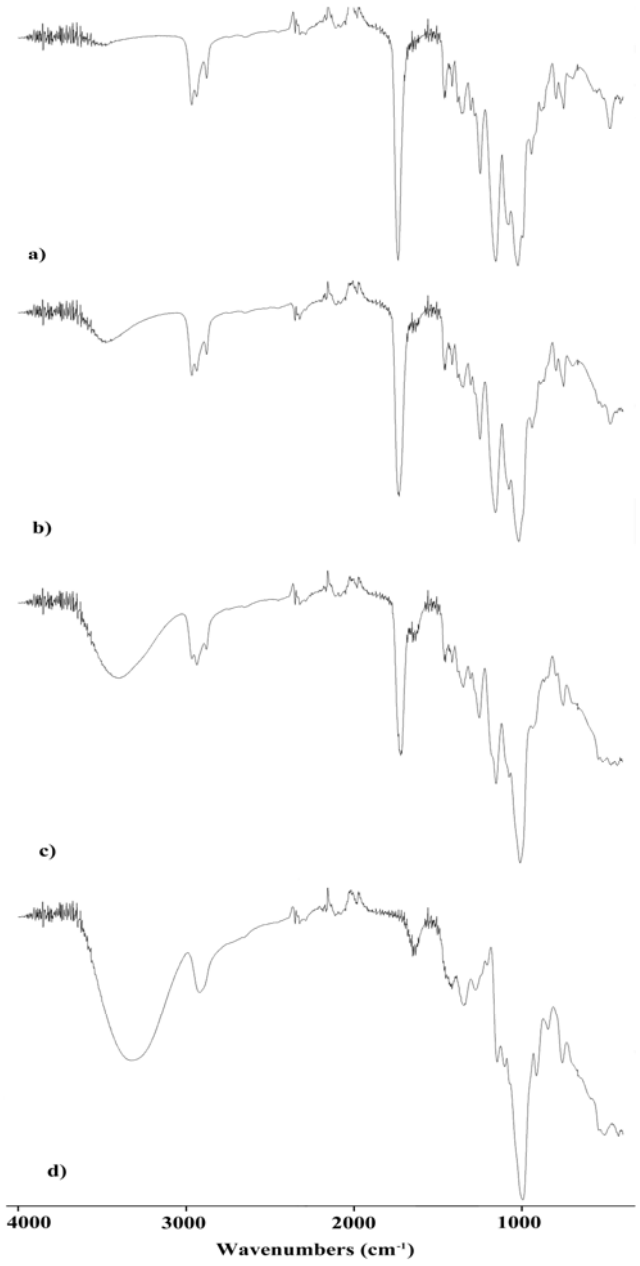


Figure 2. FTIR spectra of dextran butyrate DS.3 (a), dextran butyrate DS.2 (b), dextran butyrate DS.1 (c) and dextran (d).

The DS of each dextran esters was calculated as the following equation:

$$DS = \frac{7x}{ny} \quad (3)$$

Where *x* corresponds to the integrated areas of all the protons from the ester group, *y* corresponds to the integrated areas of all dextran protons and *n* corresponds to the number of hydrogen atoms in each ester group.

The solubilities of the different dextran esters into several organic solvents were examined and the results are reported in Table 2. As shown in Table 2, the solubilities of the different dextran esters in organic solvents are strongly dependent on the degree of substitution (DS) and on the type of the groups which are attached. By increasing the substitution degree and the length of the chains attached onto the dextran unit, the solubility of dextran esters can be improved into non polar solvents while the solubility into water is greatly reduced.

Table 2: Solubilities of the different dextran esters in various solvents

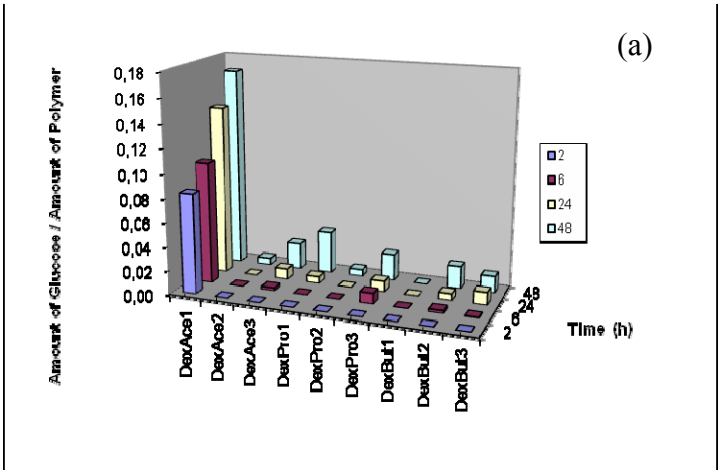
Polymer	Water	Chloroform	THF	Ethyl acetate	Acetone	2-Propanol	Methanol	DCM
Dextran Butyrate DS.3	I	S	S	S	S	PS	I	S
Dextran Butyrate DS.2	I	S	S	S	S	S	S	S
Dextran Butyrate DS.1	I	I	S	I	I	I	S	I
Dextran Propionate DS.3	I	S	S	S	S	I	PS	S
Dextran Propionate DS.2	I	S	S	S	S	S	S	S
Dextran Propionate DS.1	PS	S	S	I	I	I	S	I
Dextran Acetate DS.3	I	PS	PS	I	I	I	PS	S
Dextran Acetate DS.2	I	PS	I	I	I	I	S	PS
Dextran Acetate DS.1	S	I	I	I	I	I	S	I

S (soluble), I (insoluble). PS (particularly soluble), THF: Tetrahydrofuran; DCM: dichloromethane

The behavior of the two highly esterified dextran having a DS 2 and 3 differs strongly from esterified dextran having a DS1, allowing to conclude that the solubilities of dextran esters with a DS equal to 2 or 3 are strongly different than the dextran slightly substituted.

Enzymatic degradation of different dextran esters

The *in vitro* enzymatic degradation of different dextran esters was evaluated onto three concentrations of dextranase (Figure 3 a, b, c). As well described by several authors, the degradability of dextran derivatives by dextranase is significantly affected by the type and the extent of chemical modification [26,27] and the degree of substitution DS of dextran molecules [28]. Furthermore, the dextran esters slightly substituted (DS=1) by small chain of fatty acids, i.e. an acetate group, are degradable by the dextranase, this fact being more and more important by increasing the concentration of dextranase.



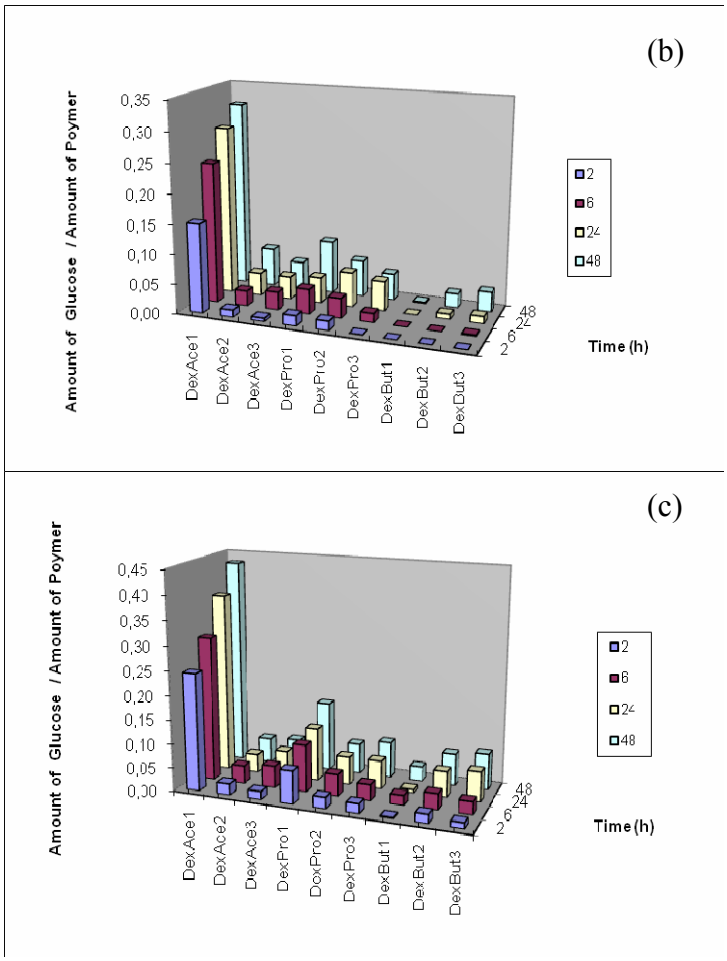


Figure 3. Enzymatic degradation of different dextran esters into different concentrations of dextranase, (a) 0.5 U/ml, (b) 5 U/ml and (c) 10 U/ml.

The study of the enzymatic degradation of the different dextran esters allowed us to conclude that the esterified dextrans degraded more rapidly as the degree of dextran substitution decreased, as the length of the attached group decreased and as the dextranase concentration increased. This enzymatic degradation study allowed us also to show that a too high substitution degree of dextran reached to a modified polysaccharide which didn't show any degradability in the concentration of dextranase used.

This fact can be explained by considering the attacking mechanism of dextranase, which needs four successive α-1,6- linked glucose units to allow the degradation of the dextran backbone as suggested by Bauer & Kesselhut [29].

Permeability test

For the reasons explained above in the section 3.2., we prepared films constituted of dextran acetate DS.1, in combination with Eudragit® RS 30D as sustained release matrix from aqueous suspension**.** The obtained data of the permeability studies of theophylline in different media are shown in Figure 4 and table 3. As it can be seen, the permeability of the drug through the different free films increased with an increase of the ratio of the dextran acetate DS.1 in the film. In addition, these films showed a high permeability in SCF medium for all of the formulations compared to the results obtained from films constituted only of Eudragit® RS 30D.

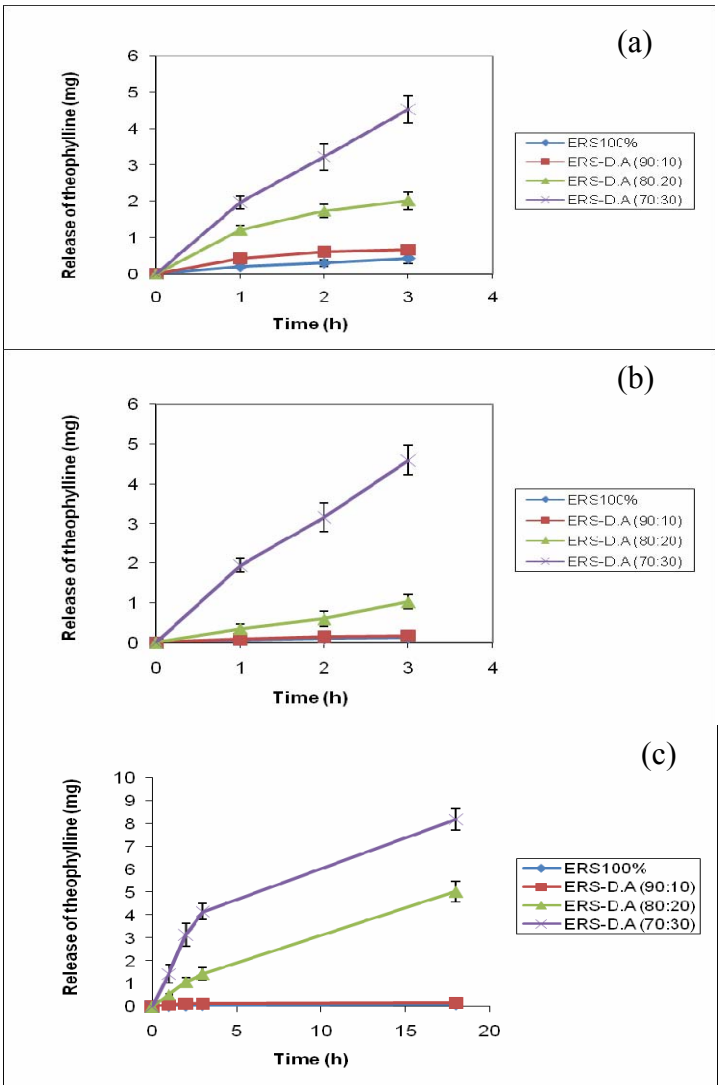


Figure 4. Permeability profiles of theophylline through free films constituted of Eudragit RS 30D - Dextran acetate (DS.1), (a) in SGF, (b) in SIF, and (c) in SCF. Error bars indicate S.D. (n = 3).

Table 3: Permeabilities of theophylline through different free films (date represent mean ± S.D.; n =3)

Formulation	P_{Theo} ($x10^{-5}$ cm/s)			
	LGS	LIS	LCS	LCS + Dextranase 0.5 U/ml
ERS 100%	1.266 ± 0.145	0.3753 ± 0.024	0.310 ± 0.034	-
ERS: Dex.Acet (DS.1) (90:10)	1.990 ± 0.045	0.510 ± 0.270	0.552 ± 0.047	0.546 ± 0.046
ERS: Dex.Acet (DS.1) (80:20)	6.006 ± 0.238	3.048 ± 0.179	14.954 ± 0.260	14.411 ± 0.416
ERS: Dex.Acet (DS.1) (70:30)	13.442 ± 0.378	13.656 ± 0.401	24.320 ± 0.332	21.566 ± 0.406

Also, as shown in Figure 5, we found little difference for the permeability of theophylline in SCF in presence or in absence of dextranase. These results can be explained by two reasons. The first one could be attributed to the too low concentration of dextranase to observe a difference between the two experiments and the second one could be attributable to the difficulty of the dextranase to reach the dextran acetate due to the presence of the non degradable, non swollen and undissolved Eudragit® RS 30D.

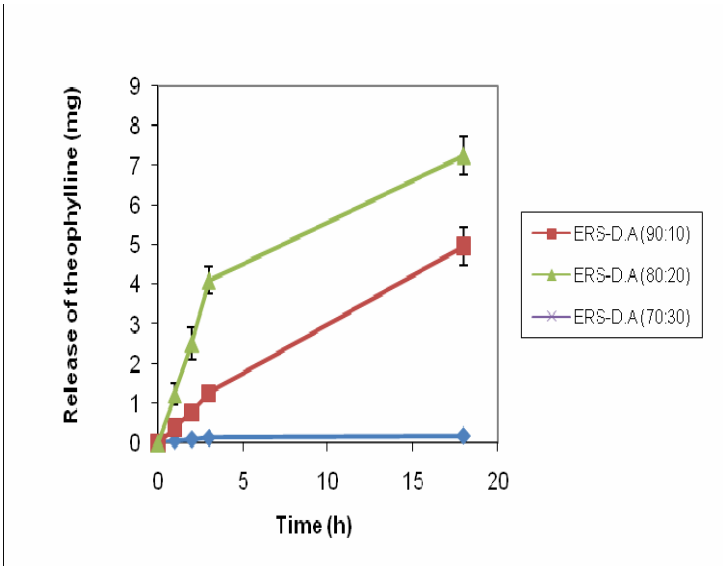


Figure 5. Permeability profiles of theophylline through free films constituted of Eudragit RS 30D - Dextran acetate (DS.1) in SCF with 0.5 U/ml of dextranase. Error bars indicate S.D. (n = 3).

The use, in the future, of polymers like cellulose derivatives which are able to swell instead of Eudragit RS 30D should allow the enzymes to degrade the dextran and then to increase the release of the drug.
Formulations containing Eudragit® RS 30D and dextran acetate, showed a higher permeability in SGF than in SIF that may be explained by the difference between the solubility of theophylline in acidic and in neutral pH which is a little lower in the latter pH. Such results should lead also, in the future, in adding an external additional enteric coating to the dosage forms (powders, tablets, capsules) in order to avoid any premature release of drug into the stomach.

Conclusion

In his study, we showed that new modified polysaccharides consisting of esterified dextrans with acid anhydrides (acetic anhydride, propionic anhydride, butyric anhydride) with three degrees of substitution can be considered as promising carriers for site specific drug delivery to the colon.
The results of this study indicate that some dextran esters i.e. dextran acetate can be enzymatically degraded whereas others more highly substituted with longer lateral chains didn't show any enzymatic degradability. The degradable modified dextans should be promising materials for coatings or as matrix system to formulate oral drug dosage forms (tablets, capsules, powders and mini granules) as carriers for site specific drug delivery to the colon.
Moreover, the combination of these dextran esters with polymethacrylate could be suitable to protect a drug

from higher regions of the gastro intestinal tract and to ensure drug release into the colon.

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