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International Journal of Pharmaceutics 158 (1997) 99–107

international journal of pharmaceutics

Interactions between food components and drugs. Part 5: Effect of acetylation and amidation of pectins on the interaction with drugs

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Received 16 June 1997; received in revised form 30 July 1997; accepted 30 July 1997

Abstract

Interactions between acetylated and amidated pectins and different drugs were studied using affinity capillary electrophoresis (ACE). It is shown that ACE is a suitable method to characterize these interactions at the molecular level. Calculating the equilibrium binding constants it was found that the structure and size of the drugs used have a stronger influence on the binding of drugs to pectins than charge and hydrophilic character. Tetracycline (TT) followed by propranolol (PP) showed the strongest interactions with the pectin derivatives studied. In contrast to these results, only the permeation of the lipophilic drug PP across artificial lipid membranes was significantly reduced by interaction with the pectin derivatives. The very low membrane transport of the hydrophilic drug TT was not influenced by the interaction. Reduction of the permeation of PP by the pectins could further influence absorption and bioavailability. © 1997 Elsevier Science B.V.

Keywords: Drugs; Propranolol; Tetracycline; Pectin; Amidation; Acetylation; Interactions; Transport; Artificial lipid membranes; Affinity capillary electrophoresis

1. Introduction

Commercial pectins are produced mainly from the residues remaining after pressing citrus fruits or apples to obtain juices. The isolated purified

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PII S0378-5173(97)00252-4

pectins consist of long chains of D-galacturonic acid (GalA) units, linked by α-1,4-glycosidic bonds. Furthermore, some rhamnose units can be present in the main chain. The macromolecular pectins can form viscous solutions or gels in aqueous media (Walter, 1990). The carboxyl groups (COOH) of GalA units are partly esterified with methanol. The degree of esterification (DE) depends upon the botanical source and the maturity of the plant material as well as on the conditions during their production.

Pectins from some sources, for instance from sugar beet (Dea and Madden, 1986), may be acetylated at the hydroxyl groups on C_2 and/or C_3 of the GalA units. Acetylation influences the various properties of pectin, for instance the gelation (Pippen et al., 1950), the interaction with metal cations (Kohn and Furda, 1968), and the activity of polygalacturonase (Rexová-Benková et al., 1977). Amidated pectins are produced by the action of ammonia on esterified pectins, replacing the methyl ester groups by amide groups. Amidation improves the gelling properties of low-esterified pectins (May, 1990) and influences the activities of pectolytic enzymes (Pilnik et al., 1974; Anger and Dongowski, 1988).

Pectin is used extensively in the food industry. Furthermore, it plays an important role as dietary fiber, both in an insoluble form (cell wall constituent of fruit and vegetables) and in a soluble, isolated form (Vahouny and Kritchevsky, 1986; Reiser, 1987; Kritchevsky and Bonfield, 1995). Pectin is also used in medicine and pharmacy (Ashford et al., 1993; Spiller, 1994). Depending on its structural parameters, pectin can interact with drugs under simulated conditions of the gastrointestinal tract in vitro. It has been shown that the DE or the distribution of free and esterified COOH of pectin can influence the transport or permeation of drugs (Neubert et al., 1992, 1993, 1995; Dongowski et al., 1996).

In this study, the influence of the degree of acetylation and amidation of pectin on the interaction with propranolol (PP), quinine, etilefrine, atenolol, ibuprofen, ketoprofen, salicylic acid, and tetracycline (TT) was investigated at the molecular level using affinity capillary electrophoresis (ACE). The determination of the changing mobilities of the ionic analytes in the presence of pectins reflected interactions between the drug and the pectin molecule. Some reference drugs were used in ACE experiments to compare the interactions. These were estimated according to the pectin concentration. The equilibrium constants, K, for the aggregation were calculated when possible.

Permeation experiments were carried out with PP and TT to estimate the influence of the pectin derivatives used on the transport of these different drugs across lipid membranes.

2. Materials and methods

2.1. Materials

2.1.1. Acetylated pectins

A total of 25 g pectin (DE 50.0%) was swollen (for 1 h at 50°C) in a mixture of 400 ml formamide and 200 ml pyridine whilst being stirred continuously. For acetylation, volumes of acetic anhydride, between 25 and 75 ml were added slowly. The preparations were then mixed in 3 l EtOH and repeatedly washed with acidified 90% EtOH and finally with 96% EtOH (Carson and Maclay, 1946; Kohn and Furda, 1968).

2.1.2. Amidated pectins

A total of 40 g pectin (DE 52.5%) in 240 ml cold EtOH was treated for 2 or 6 h with 160 ml aqueous NH_4OH solution (6–25%) whilst being shaken continuously. After acidification, NH_4Cl was extracted intensively with 60% EtOH (Lockwood, 1976).

2.1.3. Mechanically degradation of pectin

Fifteen grams of pectin derivatives were ground in a dry state in the presence of 1000 g porcelain balls (diameter: 10–12 mm) per vessel for 100 h in a vibration mill from Siebtechnik GmbH, Mühlheim, Germany (Bock et al., 1977).

2.1.4. Characterisation of pectin

Galacturonan was estimated by the m-hydroxydiphenyl method (Blumenkrantz and Asboe-Hansen, 1973). Methyl ester groups were analysed

using the chromotropic acid method (Bäuerle et al., 1977) and DE was also characterised titrimetrically (Anon, 1981). Intrinsic viscosity, η, was determined using an Ubbelohde viscosimeter at 25.0°C and pH 6.0 in presence of 0.05 M NaCl/0.005 M sodium oxalate. For estimation of the degree of acetylation (DAc), the hydroxamic acid method (McComb and McCready, 1957) and GLC of acetic acid after hydrolysis were applied. The degree of amidation (DAm) was calculated from the N-value obtained by the Kjeldahl method. Additionally, DAm and DE were determined according to the Food Chemical Index (Anon, 1981).

2.2. Permeation of propranolol and tetracycline

The model system (Neubert and Fürst, 1989) used consists of donor (DC) and acceptor compartments (AC) separated by an artificial lipid membrane with collodium as the matrix and dodecanol as the lipid. The in vitro permeation experiments were carried out at pH 7.2 (Sörensen phosphate buffer) and 37°C in the presence of 0.5 or 1.0% pectin (galacturonan). Propranolol and tetracycline were used in solutions of 1 mmol/l. The concentrations of the drugs were measured in the AC.

PP and TT were assayed using a HPLC system (Gynkotek, Germering, Germany) comprising a pump M480, autosampler GINA 160, Nucleosil $100C_{18}$ column (5 μ; 250 × 4.6 mm) with precolumn (mobile phase: acetonitrile, phosphoric acid, and water, 35:0.2:63.8), UV–vis diodarray detector (290 nm for PP and 352 nm for TT) and Gynkosoft datasystem.

2.3. Affinity capillary electrophoresis

2.3.1. Chemicals

Propranolol hydrochloride, quinine hydrochloride, etilefrine hydrochloride, atenolol, tetracycline hydrochloride, ibuprofen, ketoprofen and salicylic acid were purchased from COM-Pharmahandel GmbH, Hamburg, Germany. The model samples (1 mmol/l) were prepared by dissolving the analytically pure substances in distilled water. The buffer was prepared from reagent grade dipotassium hydrogenphosphate and potassium dihydrogenphosphate supplied by Merck, Darmstadt, Germany in concentrations of 50 mM and pH 7.4 with distilled water. The pectins were dissolved in buffer, heated up to 70°C and diluted to several concentrations. All solutions and samples were filtered through a membrane filter of 0.45 μm pore size and degassed ultrasonically before running.

2.3.2. Apparatus and methods

A Hewlett-Packard ^{3D}CE system (Waldbronn, Germany) with a one-column diode array detector (190–600 nm) was used. Fused-silica capillary 645(560) × 0.05 mm (extended lightpath) was obtained by Hewlett Packard, Waldbronn, Germany. The capillary was preconditioned for 15 min with 1 M NaOH before the first run and for 3 min with 0.1 M NaOH and 3 min with buffer prior to each run. The separation conditions used were as follows: 30 kV voltage (inlet), 250 mbar s pressure injection and 25°C capillary temperature. Detection took place at cathodic end at 200 and 220 nm. DMSO (100 μl/25 ml) was added to the samples to determine the electroosmotic flow (EOF).

3. Results and discussion

3.1. Pectin derivatives

The DAc of the prepared derivatives was between 13.5 and 55.0% (Table 1). The DAc is defined as 100% if all secondary hydroxyl groups at the C_2 and C_3 of each GalA unit are acetylated. During acetylation, the DE was hardly changed. The intrinsic viscosity, η, which corresponds to the molecular weight (Anger and Dongowski, 1988; Walter, 1990), was only decreased in the case of the highest acetylated derivative. The DAm was between 10 and 38% (Table 2). The sum of DE and DAc is constant in the prepared amidated pectin series and therefore, only a change of ester methoxyl groups by amide groups takes place during amidation. Mechanically degradation of the pectin derivatives resulted in no alterations of DAc or DAm (Dongowski and

Table 1
Preparation and characterization of acetylated pectins

Pectin derivative	Preparation	Characterization				
	acetanhydride (ml)*	Galacturonan (%)	DE (%)	Acetate (%)	DAc (%)	Intrinsic viscosity (ml/g)**
AC-1	25	75.5	49.2	6.9	13.5	436
AC-2	50	76.6	47.7	13.7	26.0	410
AC-3	60	69.4	48.2	16.0	34.0	418
AC-4	75	66.5	47.9	18.9	42.0	435
AC-5	100	64.5	48.6	24.2	55.0	290
AC-2D	50	65.9	47.7	11.8	26.0	15.2
AC-3D	60	59.9	48.1	13.8	34.0	15.4

*Acetanhydride/25 g pectin.
**ml/g galacturonan.
D, 100 h mechanolytically degraded.
DAc, degree of acetylation.
DE, degree of esterification.

Bock, 1989). The intrinsic viscosity, η, was reduced to less than 20 ml/g galacturonan after a mechanically degradations time of 100 h. In mechanically degraded pectin derivatives, the content of ash increased to more than 10% as a result of porcelain dust (Table 1 and Table 2).

3.2. Affinity capillary electrophoresis

The method of affinity capillary electrophoresis (Avila et al., 1993; Gomez et al., 1994) was used to study interactions between pectins and drugs. The principle of this method is to measure the change in electrophoretic mobility of the drug caused by aggregation with pectins. The electrophoretic mobility, μ_D, of a molecule in free solution is proportional to its electrical charge, q, and inversely proportional to the molecular mass, M_D. This relationship can be described by the approximate form $\mu_D = Cq/(M_D)^{2/3}$, where C is a constant. If the drug binds to a pectin molecule, P, a change in μ occurs due to the change in mass to M_{D+P}. In these experiments there is only a slight change in charge because of the chosen pH value of 7.4. At this pH, the net charge of pectin is approximately zero. Therefore, the determination of changing mobilities of ionic analytes in the presence of pectins reflected interactions between the drugs and pectins. Analysis of the change in mobility ($\Delta\mu = \mu_{[P]} - \mu_D$), as a function of the concentration of pectin $[P]$ yields an equilibrium constant K, where μ_D is the mobility in the absence of pectin and $\mu_{[P]}$ is the measured mobility at pectin concentration $[P]$ with reference to the content of anhydrogalacturonan. The mathematical descriptions are based on the aggregation model which was described previously (Schwarz et al., 1996).

$$K[P]^m = \frac{\mu_D - \mu_{[P]}}{\mu_{[P]} - \mu_{DP}} \quad (1)$$

μ_{DP} represent the mobility of the drug-pectin-complex and is derived from the maximum peak shift. The stoichiometric coefficient, m, is unknown. For the determination of binding constants, this equation can be transformed to:

$$\frac{\mu_{[P]} - \mu_D}{[P]^m} = K(\mu_{DP} - \mu_D) - K(\mu_{[P]} - \mu_D) \quad (2)$$

and to

$$\mu_{[P]} = \mu_D + \frac{(\mu_{DP} - \mu_D)}{1/[P]^m K} + 1 \quad (3)$$

Table 2
Preparation and characterization of amidated pectins

Pectin derivative	Preparation		Characterization				
	NH_4OH (%)*	Time (h)	Galacturonan (%)	DE (%)	DAm (%)	Sum DE+ DAm (%)	Intrinsic viscosity (ml/g)**
AM-0	0.0	—	74.1	52.2	0.0	52.2	342.0
AM-1	6.3	2	72.0	39.3	10.3	49.6	353.0
AM-2	12.5	2	72.2	28.7	20.2	48.9	309.0
AM-3	25.0	2	71.8	19.4	33.5	52.9	336.0
AM-4	25.0	6	72.2	13.4	38.1	51.5	326.0
AM-2D	12.5	2	49.1	28.7	20.2	48.9	15.3
AM-4D	25.0	6	56.2	13.4	38.2	51.6	14.7

*NH_4OH concentration (160 ml/40 g pectin).
**ml/g galacturonan.
D, 100 h mechanolytically degraded.
DAm, degree of amidation.
DE, degree of esterification.

Eq. (2) gives a linear plot, where K can be determined from the gradient. Because of high viscosity, native pectins cannot be used as a background electrolyte in ACE. For that reason, only mechanically degraded pectins (AC-2D, AC-3D, AM-2D, AM-4D) were used.

Fig. 1 shows the μ values of different drugs with AC-3D. All other pectins show similar behaviour. The cationic drugs etilefrine, atenolol and quinine and the anionic salicylic acid, ketoprofen and ibuprofen (not shown in Fig. 1) were not influenced by increasing amounts of pectin. There is a weak decrease in mobility of PP but a significant change in mobility of TT. Fig. 2 shows a comparison of PP mobilities for all tested pectins. There is the following tendency for the strength of interactions:

$$AM\text{-}2D \leq AM\text{-}4D < AD\text{-}2D < AD\text{-}3D$$

This agrees with the result found in the permeation experiments. The equilibrium constants of PP were too small ($K < 0.1$ (l/mol)) to be determined by the ACE method.

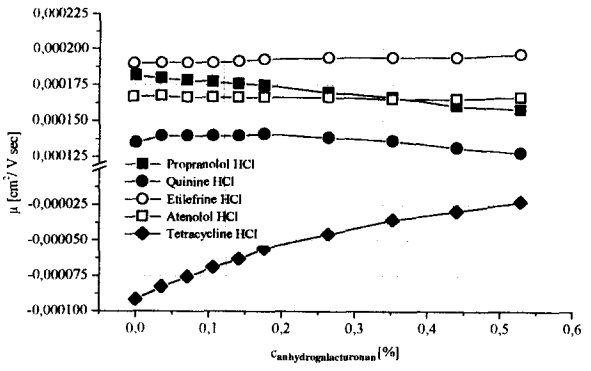

Fig. 1. The relationship between electrophoretic ionic mobility μ of drugs and pectin concentration (AC-3D), (phosphate buffer, pH 7.4, 50 mmol).

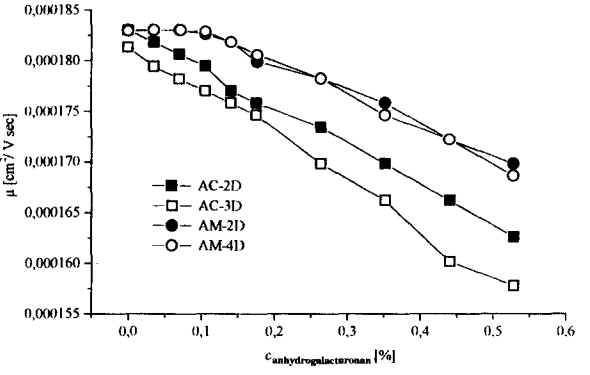

Fig. 2. Electrophoretic ionic mobility μ of propranolol hydrochloride in the presence of various pectins (phosphate buffer, pH 7.4, 50 mmol).

Table 3
Equilibrium constants of tetracycline HCl to mechanolytically degraded pectins

Pectin preparation	K [l/mol] Eq. (1)	K [l/mol] Eq. (2)	K [l/mol] Eq. (3)
AC-2D	36.1 ± 1.23	36.66 ± 0.29	29.06 ± 2.01
AC-3D	32.5 ± 3.59	31.25 ± 1.62	33.62 ± 2.07
AM-2D	26.3 ± 0.96	27.19 ± 1.04	31.45 ± 1.96
AM-4D	25.2 ± 2.28	26.85 ± 0.97	37.32 ± 3.75
	μ_{DP} [cm^2/Vs]	μ_{DP} [cm^2/Vs]	μ_{DP} [cm^2/Vs]
AC-2D	$4.37 \times 10^{-5} \pm 2.73 \times 10^{-6}$	$4.27 \times 10^{-5} \pm 3.5 \times 10^{-7}$	$6.76 \times 10^{-5} \pm 1.3 \times 10^{-5}$
AC-3D	$4.80 \times 10^{-5} \pm 1.04 \times 10^{-5}$	$5.16 \times 10^{-5} \pm 2.2 \times 10^{-7}$	$4.34 \times 10^{-5} \pm 0.9 \times 10^{-5}$
AM-2D	$5.60 \times 10^{-5} \pm 3.53 \times 10^{-6}$	$5.27 \times 10^{-5} \pm 3.7 \times 10^{-7}$	$3.68 \times 10^{-5} \pm 0.84 \times 10^{-5}$
AM-4D	$4.97 \times 10^{-5} \pm 8.52 \times 10^{-6}$	$5.26 \times 10^{-5} \pm 4.8 \times 10^{-7}$	$1.26 \times 10^{-5} \pm 1.2 \times 10^{-5}$

By comparing TT mobilities for the different pectins the following tendency in interaction strength was observed:

AM-4D < AM-2D < AC-3D < AC-2D

Assuming a 1:1 interaction between TT and pectins (stoichiometric coefficient $m = 1$), equilibrium constants can be determined (Table 3). There is a good agreement of the values determined from Eq. (1) and Eq. (2). The stoichiometric parameters for the aggregation of drug and pectin molecules are unknown. To all appearances drug-pectin-complexes with different stoichiometry contribute to measured mobility ($\mu = x\,\mu_D + y\,\mu_{DP} + \ldots + z\,\mu_{DP}m$).

This equation can be solved by assuming that the drug has only a slight influence on mobility of the pectin. By using Eq. (1) and assuming that $\mu_{DP} \approx 0$ the K value for AC-2D is 18.1 ± 8.9 (l/mol) and $m = 1.523 \pm 0.1544$.

3.3. Permeation

The influence of the pectin derivatives used on the permeation of the lipophilic drug PP and the hydrophilic TT which showed interactions as indicated by ACE, were achieved using a model membrane.

3.3.1. Influence of acetylated and amidated pectins on the permeation of propranolol

In the absence of pectin derivatives, 26.22% of the drug permeated the membrane in 3 h under the chosen in vitro conditions.

In presence of 0.5% galacturonan, with increasing degrees of acetylation, the transport of PP across the membrane decreased (Fig. 3). Whereas pectin with a DAc of 13.5% reduced the permeation to about 20%, the pectin with the highest

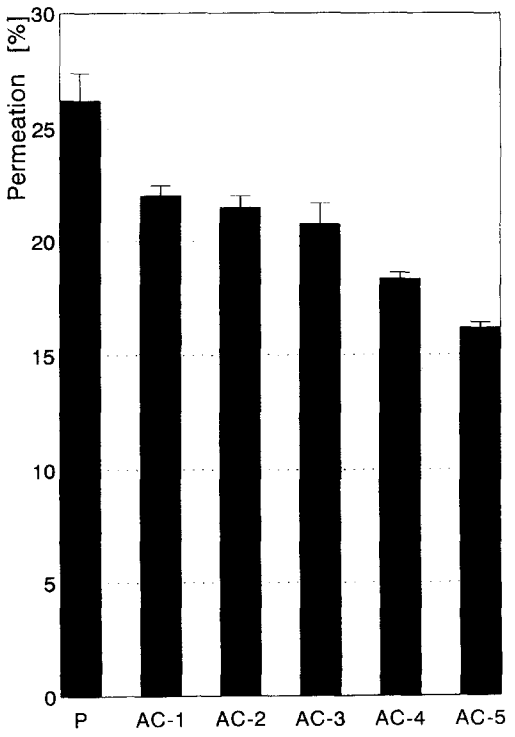

Fig. 3. Influence of acetylated pectins (AC-1–AC-5) on the permeation of 1 mmol/l propranolol across artificial lipid membranes (3 h; 37°C; 0.5% galacturonan) (P = experiment with propranolol in the absence of pectin).

Table 4
Effect of mechanolytically degraded pectin derivatives on the transport of propranolol (3 h; 37°C; pH 7.2; 0.5% galacturonan)

Pectin preparation	Permeation of propranolol (%)
AC-2D	20.54 ± 0.85
AC-3D	20.45 ± 0.98
AM-2D	20.47 ± 0.38
AM-4D	19.91 ± 0.22

Mean ± S.D.; $n = 3-4$.

DAc allowed only the transport of about 16%. The effect of acetylated pectin on the transport of PP was higher if the concentration was increased to 1% galacturonan. At this higher concentration, AC-1 and AC-2 diminished the permeation to 19.74 ± 0.62 and 19.60 ± 0.27%, respectively. In addition to the degree of derivation, the viscosity of the medium, caused by the polysaccharide, influences the permeation of the drug. Depolymerization of acetylated pectin (by mechanically degradation) is related to a decrease in the interaction (Table 4).

It was not possible to find any influence of the degree of amidation of pectin on the permeation of PP. Pectins with a DAm between 0 and 38% exhibited the same effect on the transport of the drug (Fig. 4). A permeation between 18.4 and 19.6% of PP after 3 h was measured in all cases. All pectins of this series had the same portion of free COOH. Therefore, amidated and methanol esterified pectins act to the same extent on the degree of permeation across artificial lipid membranes. Mechanolytically degraded amidated pectins were less effective in decreasing drug transport than the macromolecular preparations (Table 4).

3.3.2. Influence of acetylated and amidated pectins on the permeation of tetracycline

In contrast to PP, only 0.5% of the hydrophilic drug, TT, was transported through the artificial lipid membranes (see Fig. 5). The permeation of TT was not influenced by the acetylated and the amidated pectin derivatives.

The strong binding of TT to pectin derivatives as indicated by ACE has no relevance to the membrane transport of TT. When the extent as well as the rate of the permeation of the drug across lipid membranes is very low (e.g. it is the rate limiting step of the absorption process) the binding of the drug to biological macromolecules appears not to influence physiological processes such as membrane transport and bioavailability, respectively.

4. Conclusions

The present study shows that it is possible to characterize interactions between drugs and pectins on the molecular level using ACE. Structure and size have stronger influence on the binding of the drugs to macromolecular biopolymers such as the pectins studied, than the charge and the hydrophilic character of the drugs. A mathematical description of interactions by determination of equilibrium constants is possible if there are strong changes ($K > 1$ l/mol) in mobility of drug as influenced by galacturonan concentration.

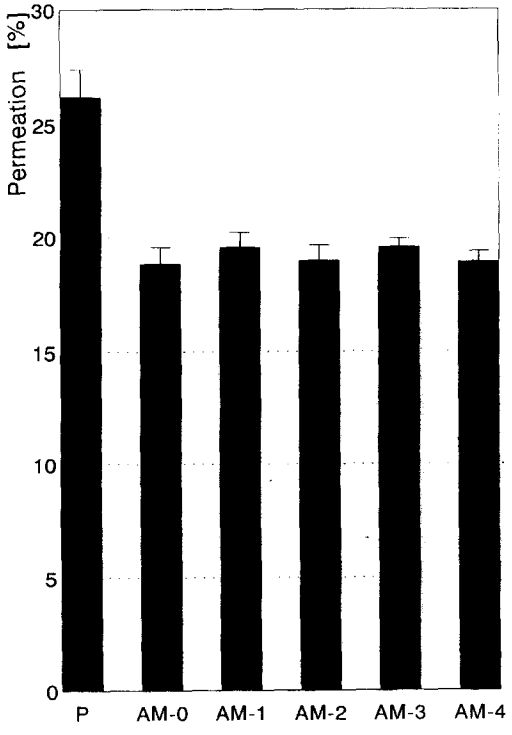

Fig. 4. Influence of amidated pectins (AM-0–AM-4) on the permeation of 1 mmol/l propranolol across artificial lipid membranes (3 h; 37°C; 0.5% galacturonan) (P = experiment with propranolol in the absence of pectin).

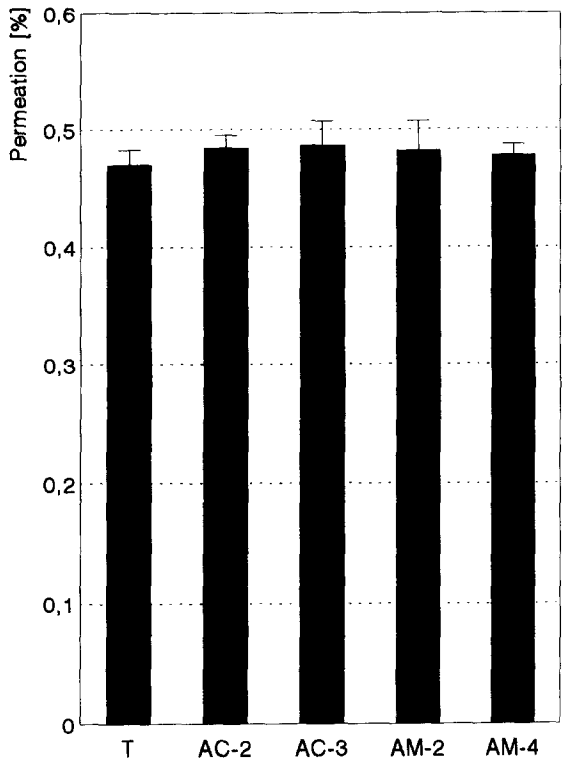

Fig. 5. Influence of acetylated (AC-2 and AC-3) and amidated (AM-2 and AM-4) pectins on the permeation of 1 mmol/l tetracycline across artificial lipid membranes (3 h; 37°C; 0.5% galacturonan) (T = experiment with tetracycline in the absence of pectin).

The relevance of the results obtained by ACE was investigated in terms of membrane permeation. It was found that the permeation of the highly hydrophilic drug TT was not influenced by the pectin derivatives used. In contrast, the permeation of the lipophilic drug, PP, was significantly decreased by interactions with pectin derivatives. These interactions may further influence physiological processes such as absorption and bioavailability.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (DFG) project NE 427/4-1 and GRK 134/1-96.

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