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in vitro and *in silico* determination of oral, jejunum and Caco-2 human absorption of fatty acids and polyphenols. Micellar liquid chromatography

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ABSTRACT

In this investigation chosen saturated, mono- and polyunsaturated fatty acids as well as polyphenols have been analyzed.

The main aim of this study was to determine oral, jejunum and Caco-2 human absorption of chosen fatty acids and polyphenols using *in vitro* and *in silico* methods. For *in vitro* determination of human drug absorption, the usefulness of Micellar Liquid Chromatography (MLC) with mobile phases containing different surfactants (including Brij35–Biopartitioning Micellar Chromatography (BMC)) has been confirmed.

On the basis of Foley's equation, $1/k$ vs. C_M correlations for the tested compounds have been done. Satisfactory linearity of the relationships was found over the whole eluents composition range studied with R^2 approximately 0.99 in each case. Moreover, the analyte–micelle association constants (K_{ma}) from Foley's equation have been compared for different micellar environments, containing Brij35, SDS and CTAB as a main component of micellar mobile phases. Completely new models describing human oral as well as Caco-2 and jejunum absorption have been constructed and compared with the cited models. These models are based on the Abraham descriptors and lipophilicity parameters as well as steric descriptors. Furthermore, many different correlations between physicochemical parameters and human intestinal absorption have been done, *e.g.* the correlation between human jejunum permeability estimated *in silico* and received using LSER parameters was excellent (R^2 nearly 0.99).

Chromatographic parameters have been collated with steric, electronic and physicochemical ones using QRAR (*Quantitative Retention – Activity Relationships*) and QSAR (*Quantitative Structure – Activity Relationships*) models. Moreover, retention BMC data have been compared with lipophilicity parameter log $P_{o/w}$ (n-octanol–water partition coefficient). The influence of lipophilicity on oral absorption (%) has been checked. The correlation between predicted oral absorption (%) and log $P_{o/w}$ has been done. Obtained R^2 was 0.82.

On the basis of chromatographic, lipophilicity, steric and different physicochemical parameters, the principal components analysis (PCA) has been done.

1. Introduction

Micellar chromatography is useful especially in describing many physiological and biopartitioning processes in a living organism. Drugs and other biologically active compounds are the main objects of interest in this technique. In our investigation fatty acids and polyphenols have been studied because of the important role played in the human body. Fatty acids are aliphatic monocarboxylic acids which act as building blocks of lipids. There exist long chain fatty acids (LCFAs) having aliphatic chains of 16 or more carbons which play an important role of nutritive energy source and are necessary for human health [1]. This group of fatty acids

Abbreviations: ALA, α-linolenic acid; GLA, γ-linolenic acid; LA, linoleic acid; CGA, chlorogenic acid; HCA, hydroxycinnamic acid; OA, oleic acid; SDA, stearidonic acid; SA, stearic acid; CA, caprylic acid; DA, decanoic acid; LUA, lauric acid; MA, myristic acid; PA, palmitic acid; EA, erucic acid; POA, palmitoleic acid; BMC, Biopartitioning Micellar Chromatography; MLC, Micellar Liquid Chromatography; SDS, sodium dodecyl sulfate; CTAB, cetyltrimethylammonium bromide; Brij35, polioxyethylene (23) lauryl ether; THF, tetrahydrofuran

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can include the polyunsaturated fatty acids (PUFAs) which contain two or more double bonds (LCPUFAs–long chain polyunsaturated fatty acids). Since humans cannot synthesize double bonds at position 6 or lower, they must be obtained from the diet. Fatty acids with shorter carbon chain (8–14 carbon atoms) are called medium chain fatty acids (MCFAs). Triacylglycerols containing fatty acids with more than 12 carbon atoms must be hydrolyzed in the intestinal lumen before absorption. Therefore, the MCFAs can directly be absorbed without hydrolysis and preferentially transported through the portal venous system to the liver [1].

The oral route is preferred for routine administration of bioactive compounds required to have systemic actions, as it is the most convenient and cost-effective. In order for a drug to reach the systemic circulation and its site of action, it must have chemical and physical properties that allow it to withstand the hostile environment within the gut lumen [2]. It is commonly known that the intestinal mucosa is the major oral absorption barrier. However, drugs molecules can be available for absorption if they have appropriate lipophilicity and solubility characteristics. The most frequently used lipophilicity factor is n-octanol–water partition coefficient ($\log P_{o/w}$) which is the key factor in oral drug absorption. Transport across the intestinal epithelium may be in progress *via* paracellular or transcellular route. Transcellular transport of compounds may be passive or mediated by specific transporters at apical and basolateral membrane surfaces. Different drugs are absorbed by passive diffusion mechanism. Besides appropriate lipophilicity and solubility features, compounds which may cross the intestinal mucosa *via* passive diffusion mechanism, require also appropriate physicochemical properties such as molecular size, charge and hydrogen bond potential. They are important in order to allow passage through both the apical and basolateral plasma membranes of the enterocyte [2].

Today pharmacological drugs investigations comprise extremely important knowledge area, *sensu largo*. In the early stage of drug discovery, the pharmacokinetic studies have traditionally been conducted in living systems such as mice, rabbits, dogs, *etc*., but this methodology is expensive and time consuming [3]. For ethical as well as economical reasons, the necessity of development of *in vitro* and *in silico* investigations is emphasized in order to avoid or at least reduce animal experiments [3,4].

Therefore, many different *in vitro* models for estimation of drug absorption and transport in the intestinal epithelium are examined. Recently, the commonly utilized model is Caco-2, a colorectal adenocarcinoma cell line of human origin. The Caco-2 cell monolayers are generally accepted as a primary absorption screening tool in several pharmaceutical companies [3]. The Caco-2 cell line is nowadays very popular in the wide range of possible applications such as *in vivo* prediction of different compounds absorption [5–7] or *in vitro* permeability measurements [8–9]. The main oral drug absorption barrier is the intestinal mucosa where drugs are absorbed by the passive diffusion mechanism. The Caco-2 cell line which is functionally similar to intestinal epithelium *in vivo* naturally occurring, is now commonly used model system especially in pharmaceutical investigations. In this study the Caco-2 cell culture model has been used for the *in silico* characterization of intestinal permeability properties of fatty acids and polyphenols. Then, drug transport in Caco-2 monolayers has been compared with other drug absorption ways. There do not exist any publications focusing on evaluation of the three different types of drug absorptions: jejunum, oral and Caco-2, using chromatographic as well as *in silico* methods, by then.

For *in vitro* drug absorption estimation, Biopartitioning Micellar Chromatography (BMC), which is one of the types of Micellar Liquid Chromatography (MLC), has been used. MLC is a mode of conventional reversed-phase liquid chromatography which uses a surfactant solution (anionic, cationic or non-ionic) above the critical micellar concentration (CMC) as a mobile phase [10,11]. The retention of compounds in MLC depends on the type of interaction (electrostatic and/or hydrophobic) with the micelles and with a surfactant-modified stationary phase [11–14].

BMC uses micellar mobile phases of non-ionic polioxyethylene (23) lauryl ether, Brij35 and C18 reversed stationary phase, under adequate experimental conditions. This technique can be useful in describing the biological behavior of different kinds of organic compounds and can mimic many biological processes such as blood–brain barrier penetration, skin permeability, intestinal absorption and drug partitioning process in biological systems [15–20]. BMC is generally treated like a useful model for describing many important biological processes in a living organism [21–23].

The main aim in this investigation was to check how commonly existing empirical models based on the retention data, lipophilicity parameters, and Abraham descriptors can estimate the oral drug absorption and to construct completely new models of oral, jejunum and Caco-2 human absorption of fatty acids and polyphenols. Therefore, for the tested compounds, human jejunum absorption, absorption rate and Caco-2 permeability have been estimated using QRAR as well as QSAR models. Micellar Liquid Chromatography has been used as an *in vitro* technique for the determination of the absorption rate. QSAR models were based on the *in silico* approach using computational methods for the estimation of the absorption rate, jejunum as well as Caco-2 human absorption of the studied compounds.

MLC systems with two different micellar mobile phases containing cationic cetyltrimethylammonium bromide (CTAB) as well as anionic sodium dodecyl sulfate (SDS) have been compared with BMC systems to check how strong interactions between tested compounds and proper micellar medium are. On the basis of Foley's equation, important physicochemical parameters have been calculated such as analyte–micelle association constant (K_{ma}) and partition coefficient of analyte between stationary phase and water (P_{sw}–hydrophobicity descriptor).

Furthermore, the principal components analysis (PCA) has been done on the basis of chromatographic, lipophilicity, steric and electronic parameters. It was important to establish the relationships between different variables. Tested compounds have been divided depending on the structure into four groups: saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids and polyphenols. It was necessary to check how each of the tested compounds behaves within own group. Moreover, to characterize micellar systems used and to compare the systems with different physicochemical and biological parameters, the Linear Solvation Energy Relationships (LSER) of the Abraham model [24–28] was applied.

2. Experimental

The investigated substances have been presented in Table 1. Many micellar systems have been tested using surfactants with different structures: cationic CTAB, anionic SDS and non-ionic Brij35. In our investigation the following systems have been checked (Table 2).

Micellar mobile phases were prepared by dissolving proper surfactant (for HPLC, Merck, Darmstadt, Germany) in buffered solutions at pH 7.4 (which correspond to blood pH of humans and therefore is considered as the most physiological for humans) to get a final surfactant concentrations of 0.04 M, 0.06 M, 0.08 M, 0.1 M and 0.12 M. The buffer was prepared from 0.02 M Na_2HPO_4 and 0.01 M citric acid (analytical reagents, Merck, Darmstadt, Germany) mixed together and vacuum-filtered through 0.45 μm membrane filter before use. The pH value of the buffer was measured before the preparation of the mobile phases addition

Table 1
Structures of the tested compounds.

saturated fatty acids	unsaturated fatty acids				polyphenols
	mono-	di-	tri-	poly-	
caprylic	oleic	linoleic	α-linolenic	stearidonic	2-hydroxycinnamic acid (o-coumaric acid)
decanoic					3-hydroxycinnamic acid (m-coumaric acid)
lauric	erucic				4-hydroxycinnamic acid (p-coumaric acid)
myristic			γ-linolenic		
palmitic	palmitoleic				chlorogenic acid (ester of caffeic acid and (-)-quinic acid)
stearic					

Table 2
Tested MLC and BMC systems.

Stationary phase	Mobile phase		Component content		Surfactant character
	Surfactant	Organic modifier	Surfactant (M)	Organic modifier (% v/v)	
CN F_{254} 10 × 10	SDS (sodium dodecyl sulfate)	Tetrahydrofuran acetone 1,4-dioxane	0.04, 0.06, 0.08, 0.1, 0.12	20	Anionic
CN F_{254} 10 × 10	CTAB (cetyltrimethylammonium bromide)	Acetonitrile	0.04, 0.06, 0.08, 0.1, 0.12	20	Cationic
CN F_{254} 10 × 10	Brij35 (polioxyethylene (23) lauryl ether)	Acetonitrile	0.04, 0.06, 0.08, 0.1, 0.12	0, 5, 10, 15, 20	Non-ionic

of organic modifier. Distilled water was obtained from Direct-Q apparatus (Millipore). Micellar mobile phases were degassed in the ultrasonic bath for about two minutes before use. As an organic modifier proper solvents have been utilized (all for HPLC, Merck, Darmastadt, Germany). All the chromatographic measurements have been led on the cyano-modified silica plates (Merck, Darmastadt, Germany). Chromatograms visualization has been done after plates air-drying using iodine vapor.

3. Results and discussion

3.1. Micellar chromatography of compounds tested

The retention factors were measured for all the tested MLC as well as BMC chromatographic systems (Table 2). In order to predict possible interactions of the investigated solutes in micellar environment, retention factors should be extrapolated to pure water using Foley's equation [29]:

$$\frac{1}{k}=\frac{K_{sm}C_M}{k_s}+\frac{1}{k_s} \quad (1)$$

where C_M is the concentration of micelles ($C_M=C-CMC$, where: C is the total surfactant concentration, CMC is the critical micelle concentration), K_{sm} is the analyte–micelle stability constant, and k_s is the retention factor extrapolated to pure water.

Satisfactory linearity of the relationships was found over the whole eluents composition range studied with the coefficient of determination, R^2 approximately 0.99 in each case.

In order to check what interactions of the tested compounds in each micellar environment are like, some significant physicochemical parameters can be calculated according to modified Foley's equation (Eq. (2)) [29]:

$$\frac{1}{k}=\frac{K_{ma}C_M}{P_{sw}\Phi}+\frac{1}{P_{sw}\Phi} \quad (2)$$

where K_{ma} is the analyte–micelle association constant, P_{sw} is the partition coefficient of analyte between stationary phase and

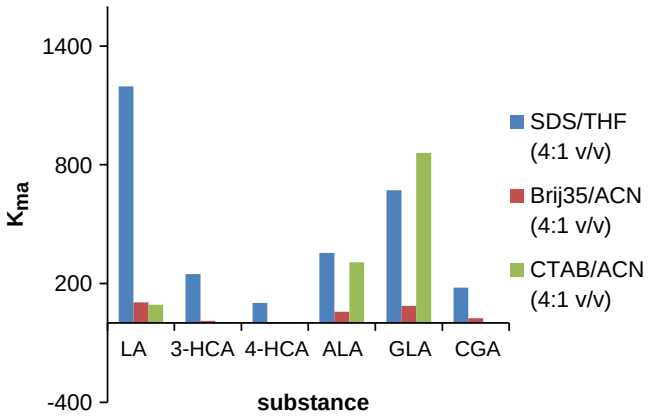

Fig. 1. K_{ma} values obtained for chosen compounds in different micellar systems.

water; hydrophobicity descriptor, Φ is the volume ratio of stationary phase to volume of mobile phase.

The most important parameters are the analyte–micelle association constant (K_{ma}) and partition coefficient of analyte between stationary phase and water (P_{sw}). K_{ma} value can give us some information about existing interactions between analyte and micelle. Since micelle is commonly treated like a simple cell membrane model, the K_{ma} parameter can describe possible interactions between cell membrane (and the other biological barriers *e.g.* intestinal epithelium) and the tested substances.

K_{ma} values obtained for chosen compounds in micellar systems containing ionic SDS and CTAB as well as non-ionic Brij35, have been compared in Fig. 1. As we can observe in Fig. 1, chosen acids interact the strongest with SDS micelle in mobile phase containing THF as an organic modifier, except for GLA which interacts stronger with cationic CTAB. It may result from the fact that GLA as a structural isomer in relation to ALA, is the most lipophilic among the presented compounds. Retention of many different compounds in MLC depends on possible interactions with micelles and surfactant-modified stationary phase [30,31]. Structures of substances and their physicochemical properties can determine their biological activity. One of the descriptor which can describe that biological activity is a lipophilicity parameter log $P_{o/w}$ which can be determined theoretically on the basis of structural parameters of solutes. Therefore, the next step of this investigation was to compare extrapolated to pure water logarithms of retention factors for compounds studied in BMC systems ($\log k_{sBMC(exp)}$) with log $P_{o/w}$ obtained using ACD/Percepta software. For the tested substances there exist very good correlation with $R^2 > 0.94$. It could suggest that the above mentioned equation (Eq. (2)) is appropriate to describe possible interactions in micellar environment. Good correlation obtained between $\log k_{sBMC(exp)}$ and log $P_{o/w}$ can suggest a large similarity between BMC and the n-octanol/water system.

3.2. BMC for predicting oral absorption

As it was previously mentioned, compounds may cross the intestinal epithelium by paracellular or transcellular routes. All the compounds tested may be transported across the intestinal epithelium *via* transcellular pathway by the passive transport mechanism. Biological activity descriptors such as intestinal absorption can be determined by means of *in silico* and *in vitro* experiments for systems imitating biological membranes. One of them is the BMC system which besides many other different things could also mimic the environment of drug biological partitioning. The retention in BMC depends on hydrophobic, electronic as well as steric properties of the tested compounds. These features of compounds also determine their passive permeability across cell membranes [11].

In order to predict oral drug absorption for the compounds tested at physiological pH 7.4 the following QRAR model has been utilized [3]:

$$\text{Oral absorption } (\%) = 100k_{BMC}/[(1.0 \pm 0.3) + (1.00 \pm 0.03)k_{BMC}] \quad (3)$$

where $n=74$, $R^2=0.72$, SE$=9.8$, and $F=3174$

On the basis of Eq. (3), oral absorption for compounds studies has been predicted for BMC systems containing 0.1 M Brij35 in the micellar mobile phase (without any existence of organic modifier at pH 7.4). Obtained oral absorption (%) values have been compared with k_{BMC} values. We obtained parabolic relationship with excellent $R^2 > 0.97$. This could suggest that this model can describe properly oral absorption processes for the tested compounds and the BMC system mimics adequately drugs interactions with biomembranes. Moreover, all the chosen compounds have retention factors higher than 3. It can denote that tested compounds have high permeability and are rapidly and completely absorbed with absorption even higher than 90%. Only in the case of hydroxycinnamic acids the absorption is lower than 80%. However, compounds can differ in different properties, i.e. solubility which is a very important factor for the drug dissolution process [32].

Moreover, it was also examined how the other tested BMC systems can predict oral absorption. For this purpose oral absorption (Eq. (3)) for chosen compounds has been calculated using micellar BMC systems with different contents of organic modifier at pH 7.4. As we supposed previously, predicted on the basis of various chromatographic data oral absorption is the lower the more organic modifier mobile phase contains. Therefore, BMC systems without any amount of organic modifier describe in the most appropriate way different biological processes including oral absorption. Mobile phases containing only buffered solutions (at physiological pH 7.4) of Brij35 imitate biological systems of a living organism most reliably. Many commonly used QRAR models for predicting oral absorption can be very useful in the early stage of research on new drugs. It results from the fact that retention in BMC can be coherent with transport properties of compounds if passive diffusion is the mechanism responsible for absorption.

Besides, the influence of surfactant concentration on predicted oral absorption has been checked. For this purpose oral absorption has been calculated using retention data obtained for BMC systems containing 20% v/v of acetonitrile and different concentrations of Brij35 (80% v/v) in buffered mobile phase. Relationship between oral absorption (%) and k_{BMC} values obtained using different BMC systems is presented in Fig. 2. As we can observe, the surfactant

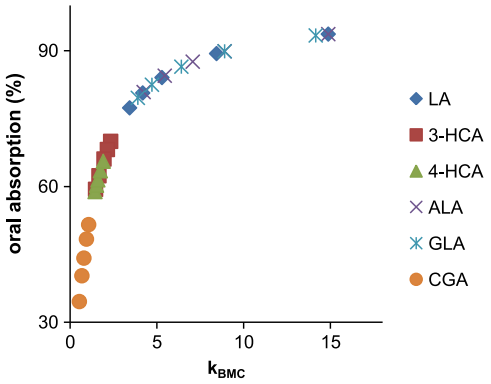

Fig. 2. Oral absorption vs. k_{BMC} values obtained for BMC systems containing 20% v/v of acetonitrile and 0.04 M, 0.06 M, 0.08 M, 0.1 M, 0.12 M Brij35 (80% v/v) in micellar mobile phases at pH 7.4.

concentration in micellar mobile phase is also an important factor for *in vitro* oral absorption determination. Moreover, the influence of lipophilicity on oral absorption (%) was checked. The correlation between predicted oral absorption (%) and log $P_{o/w}$ has been done. Obtained $R^2 > 0.82$ was satisfactorily high which means that lipophilicity is another agent impacting on oral absorption of compounds tested.

The BMC model for describing oral drug absorption can be very useful, however, there also exist a few limitations. This model does not include factors decreasing the absorption of drugs during chemical and bacterial degradation at the absorption site as well as it does not include the first pass metabolism in the intestinal cells and liver. There can also exist other mechanisms of membrane passage *via* paracellular routes or active transport which the BMC system does not concern for predicting the oral drug absorption model [3].

Table 3
The Abraham descriptors of the compounds studied.

Name	A	B	Bo	L	S	E	V
Lauric acid	0.57	0.39	0.39	6.80	0.66	0.16	1.87
Myristic acid	0.57	0.39	0.40	7.79	0.67	0.15	2.16
Palmitic acid	0.57	0.40	0.41	8.78	0.68	0.15	2.44
Stearic acid	0.57	0.41	0.41	9.77	0.68	0.15	2.72
Linoleic acid	0.57	0.53	0.54	9.75	0.88	0.46	2.63
Oleic acid	0.57	0.47	0.48	9.76	0.78	0.30	2.68
2-Hydroxycinnamic acid	1.07	0.79	0.80	6.33	1.39	1.13	1.23
3-Hydroxycinnamic acid	1.07	0.79	0.80	6.33	1.39	1.13	1.23
4-Hydroxycinnamic acid	1.07	0.79	0.80	6.33	1.39	1.13	1.23
α-Linolenic acid	0.57	0.60	0.61	9.74	0.98	0.61	2.59
γ-Linolenic acid	0.57	0.60	0.61	10.73	0.99	0.61	2.87
Chlorogenic acid	2.02	2.25	2.24	12.63	2.53	2.09	2.42
Caprylic acid	0.57	0.38	0.38	4.82	0.64	0.16	1.31
Decanoic acid	0.57	0.38	0.39	5.81	0.65	0.16	1.59
Erucic acid	0.57	0.48	0.49	11.74	0.80	0.30	3.24
Palmitoleic acid	0.57	0.46	0.47	8.77	0.77	0.31	2.39
Stearidonic acid	0.57	0.60	0.61	9.74	0.98	0.61	2.59

3.3. The LSER of Abraham for predicting human intestinal absorption

The Linear Solvation Energy Relationship (LSER) of Abraham assists in the interpretation and prediction of retention data in diverse chromatographic modes [33]. Moreover, the LSER model is utilized to characterize many biological and physicochemical processes since biological activities and chromatographic retention of solutes are based on the same basic intermolecular interaction forces, such as hydrophobic, electronic, steric effects and hydrogen bond. Setting up the relationships among these processes by LSER coefficients will be beneficial when we choose the suitably high-throughput chromatographic systems to model some biological processes well [34]. The general equation which was used is the same as that originally employed by Abraham et al. [35–39] LSER is expressed as follows:

$$SP = c + vV + sS + bB + aA + eE \quad (4)$$

where SP is the dependent solute property in a given system such as the logarithm of chromatographic retention factors (log k), the logarithm of n-octanol–water partition coefficients (log $P_{o/w}$), *etc*. In this work SP is a set of human intestinal absorption values. The independent variables are solute descriptors: V is the solute McGowan volume in units of $cm^3\ mol^{-1}/100$, S is the polarizability/dipolarity, B is the overall hydrogen-bond basicity, A is the overall hydrogen-bond acidity and E is an excess molar refraction. The coefficients: v, s, b, a, and e reflect the differences in the two phases between which the compound is transferred.

The LSER has not only been applied to characterize diverse chromatographic systems, but also utilized to study many physicochemical and biopartitioning processes such as human intestinal absorption [34,35], permeation and distribution across the BBB [36,37] and human skin permeation and partition [38].

In this study LSER was applied to characterize human intestinal absorption. The values of all the Abraham descriptors were calculated for the tested compounds using ACD/Percepta software (Table 3). The QSAR model with the Abraham descriptors can be used for prediction of human intestinal absorption (%Abs) for compounds tested. Calculated values of %Abs were obtained on the

Table 4
Chosen steric, electronic, physical and physicochemical parameters for the tested compounds.

Name	Steric desciptors				Electronic descriptor	Physical properties	Physicochemical properties	Molecular structure
	Molecular weight	Parachora [cm^3]	Molar volume [$Å^3$]	Molar refractivity	Polarizability	Melting point [°C]	*pKa*	TPSA[$Å^2$]
Lauric acid	200.32	531.36	225.77	58.68	23.34	43.2	4.95	37.3
Myristic acid	228.37	610.93	259.82	67.88	27.03	54.0	4.95	37.3
Palmitic acid	256.42	690.50	293.52	77.08	30.71	62.0	4.95	37.3
Stearic acid	284.48	770.07	327.76	86.29	34.40	72.0	4.95	37.3
Linoleic acid	280.45	744.49	312.20	88.52	34.54	−12.0	4.78	37.3
Oleic acid	282.46	757.28	320.05	87.40	34.51	16.0	4.78	37.3
2-Hydroxycinnamic acid	164.16	347.04	144.63	45.04	16.81	217.0	4.04	57.53
3-Hydroxycinnamic acid	164.16	347.04	144.56	45.04	18.07	195.0	4.38	57.53
4-Hydroxycinnamic acid	164.16	347.04	144.61	45.04	18.07	212.0	4.20	57.53
α-Linolenic acid	278.43	731.70	304.67	89.64	34.56	−11.0	4.78	37.3
γ-Linolenic acid	306.48	838.27	338.54	98.84	40.09	202.0	4.77	37.3
Chlorogenic acid	354.31	681.77	298.23	83.24	32.52	210.0	3.81	164.75
Caprylic acid	144.21	372.23	157.68	40.28	16.13	16.7	5.19	37.3
Decanoic acid	172.26	451.80	191.71	49.48	19.80	31.0	4.95	37.3
Erucic acid	338.57	916.42	388.83	105.81	41.86	33.8	4.95	37.3
Palmitoleic acid	254.41	677.71	286.04	78.20	30.84	0.0	4.99	37.3
Stearidonic acid	278.43	731.70	297.01	90.75	34.56	−57.0	4.92	37.3

The above parameters were calculated using ACD/Percepta software.

basis of different equations of Abraham et al. taken from Ref. [39]

$$\%\text{Abs} = 92.0 + 2.94\mathbf{E} + 4.10\mathbf{S} - 21.70\mathbf{A} - 21.10\mathbf{B} + 10.60\mathbf{V}$$

$$\text{where, } n = 169,\ R^2 = 0.74,\ \text{SD} = 14\%,\ \text{and } F = 93 \quad (5)$$

In the presented work [39] authors focused on analyzing the influence of strong Brønsted acids and bases. The above Eq. (5) has been reorganized making the incorporation of an indicator variable for acids with $pKa < 4.5$ and bases with $pKa > 8.5$. However, the indicator variable does not make spectacular differences in % absorption for strong Brønsted acids and bases. Different steric, electronic, physical and physicochemical parameters have been calculated using ACD/Percepta (Table 4). In our case only four among compounds tested (2-HCA, 3-HCA, 4-HCA and CGA) according to the above rules can be classified as strong Brønsted acids. Moreover, three of them (HCAs) follow the Lipiński rule of five [40] whereas CGA does not. However, any simplifications should be avoided because there exist many exceptions to this rule.

On the basis of Eq. (5), %Abs has been calculated for compounds tested. For 14 compounds among compounds tested %Abs more than 90% has been observed. Only for HCAs and CGA obtained % Abs was much lower, the lowest for CGA (only 43%). Taking into account *pKa* values and the rule indicated by Abraham et al. concerning the Brønsted acid–base theory, these compounds belong to strong Brønsted acids which is confirmed by low %Abs values. However, Abraham et al. noticed that the effect of ionizable compounds on the observed %Abs is very small. They are probably absorbed in an indirect way because of existing ionic↔neutral equilibrium which provides a pathway for ionic compounds. Acids and bases ionized will appear to permeate by order of magnitude less than the neutral compounds. This would greatly impact on the % absorption [39].

The rest of compounds tested are not strong Brønsted acids or bases. For substances which do not belong to "Brønsted compounds", the relationship between calculated %Abs and log $P_{o/w}$ has been received and is presented in Fig. 3. High value of obtained ($R^2 > 0.91$) could confirm that lipophilicity is one of the main factors determining human intestinal absorption. In the literature different relationships between permeability through any biological membrane (small intestine, skin, Caco-2, blood–brain barrier, *etc.*) and lipophilicity are widely observed [41,42]. In this work linear relationships between %Abs and log $P_{o/w}$ have been obtained, however different characters of these relationships may be noticed, *i.e.* hyperbolic, sigmoidal, parabolic or bilinear. It proves that lipophilicity influences very strongly on permeability through biological membranes in humans.

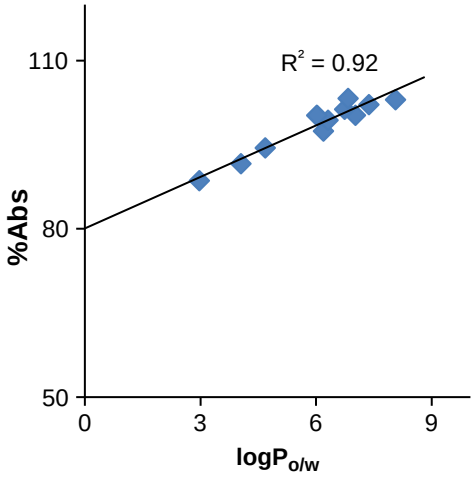

Fig. 3. Calculated %Abs vs. log $P_{o/w}$ relationships obtained for chosen compounds.

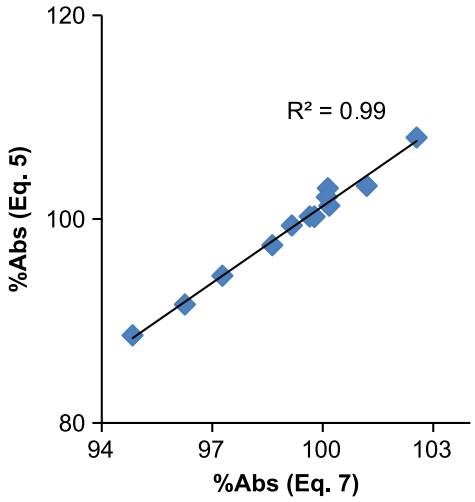

Fig. 4. Comparison between %Abs values calculated on the basis of Eq. (5) and self-constructed Eq. (7) for the tested compounds.

Zhao et al. also offered the LSER model for describing human intestinal absorption [34]. In this work %Abs values have been obtained for 169 diffusion rate-limited drugs. In the next work [43] previously described model has been refined as shown in Eq. (6) where %Abs depends on a linear combination of the descriptors through first order kinetics:

$$\text{Abs}\% = 100[1 - \exp(-10^{0.435 + 0.0848E + 0.0405S - 0.348A - 0.403B + 0.232V})]$$

$$\text{where, } n = 169,\ S = 13,\ \text{and } R^2 = 0.78 \quad (6)$$

On the basis of Eq. (6), %Abs values for tested compounds have been received. For most of the compounds %Abs was higher than 95%. Only for 2-HCA, 3-HCA, 4-HCA and CGA these values are considerably lower. However, calculated %Abs values are very similar in the above mentioned models. The relative error for % Abs between these two models for each of the compound tested was approximately 4.6%. It denotes that Eqs. (5) and (6) describe similarly and correctly the intestinal absorption of investigated substances.

Moreover, for calculating human intestinal absorption % we constructed new models for our compounds tested to compare them with the above cited models. The first one is based on the Abraham descriptors

$$\%\text{Abs} = 99.8 - 41.9\mathbf{A} - 24.7\mathbf{B} + 42.9\mathbf{S} - 17.7\mathbf{E} + 2.59\mathbf{V}$$

$$\text{where, } n = 17,\ S = 0.74,\ \text{and } R^2 = 99.9\% \quad (7)$$

The second model takes into account lipophilicity parameters as well as steric descriptor

$$\%\text{Abs} = 96.90 + 12.20\text{AC log } P - 3.20 \log P_{o/w} - 0.189\ \text{MV} \quad (8)$$

where MV is the molar volume [Å^3], $n = 17$, $S = 2.32$, $R^2 = 98.3\%$

Obtained %Abs values using these two above models have been correlated with log $P_{o/w}$ values. In both cases R^2 value was satisfactorily high (0.94 and 0.93, respectively). This indicates that our new constructed models are appropriate for the determination of human intestinal absorption of the tested saturated and unsaturated fatty acids as well as of polyphenols. Moreover, it was also examined what the differences between literature and self-constructed models are like. For this purpose, the correlation between %Abs values obtained using Eqs. (5) and (7) which both base on Abraham descriptors has been done and presented in Fig. 4. In this correlation, the previously mentioned substances which belong to strong Brønsted acids have been omitted. For the rest of compounds tested, excellent R^2 (nearly 0.99) has been received. It confirms that our model is very suitable for

describing % intestinal absorption for fatty acids not being strong Brønsted acids.

3.4. Jejunum and Caco-2 model absorption

The most commonly known cell line for prediction intestinal absorption is Caco-2 cell line which can be cultivated to spontaneously differentiate to form monolayers or polarized cells with similar functions to intestinal enterocytes. The rate of passive diffusion through the monolayer (the rate at which miscellaneous compounds cross the membrane) is characterized by Fick's law: $dm/dt = PAC$, where P is the permeability coefficient expressed in cm s^{-1}, A is the available surface area and C is the concentration of soluble drug in the donor compartment [44]. In other words, the rate of passive diffusion can be expressed in the following way: $K[A(C_1 - C_2)/D]$, where K is the diffusion constant which is related to the molecular structure, solubility and degree of ionization of tested compounds, A is the area available for diffusion, $C_1 - C_2$ is concentration gradient across the membrane and D is thickness of the membrane.

For all the tested compounds passive human jejunum (pH 6.5) and Caco-2 absorption expressed as permeability in [cm s^{-1}] as well as human intestinal absorption rate [min^{-1}] have been determined *in silico*. These values are presented in Table 5. Completely absorbed substances have high permeability in the Caco-2 monolayer, $P_{e,Caco\text{-}2} > 1 \times 10^{-6}$ cm s^{-1} whereas incompletely absorbed compounds have low permeability coefficients, $P_{e,Caco\text{-}2} < 1 \times 10^{-7}$ cm s^{-1}. For the tested fatty acids and polyphenols, there are four compounds which have high Caco-2 permeability: DA, CA, LUA, MA, therefore they are completely and fast absorbed in the Caco-2 monolayer. These substances belong to saturated fatty acids. Such similar behavior is then explicable. In addition, we can observe that ability to permeate through the Caco-2 monolayer may depend on carbon chain length because PA which contains more carbon atoms than DA, CA, LUA and MA (16 carbon atoms), is found to have lower permeability whereas SA which contains 18 carbon atoms, does not exhibit any ability to permeate through the Caco-2 monolayer. There exist three compounds which are not permeable through the Caco-2 cell line: SA, CGA and EA. Compounds that are slowly and incompletely passively absorbed distribute poorly into cell membranes. It is generally assumed that these substances are transported through the water-filled pores of the paracellular pathway across the intestinal epithelium. However, it is also possible that even very hydrophilic compounds may be transported mainly by the transcellular route [8]. All the studied compounds are transported across the intestinal epithelium by passive transcellular route and are in general lipophilic.

Table 5
Passive human jejunum and Caco-2 absorption expressed in [cm s^{-1}] and human intestinal absorption rate [min^{-1}] obtained for compounds tested.

Name	$P_{e,jejunum}$ [10^{-4} cms^{-1}]	Absorption rate, k_a [min^{-1}]	$P_{e,Caco\text{-}2}$ [10^{-6} cms^{-1}]
Lauric acid	8.49	0.058	38.0
Myristic acid	8.22	0.056	36.0
Palmitic acid	7.89	0.054	0.3
Stearic acid	7.61	0.052	0.0
Linoleic acid	7.69	0.053	0.5
Oleic acid	7.65	0.052	0.1
2-Hydroxycinnamic acid	1.24	0.008	6.0
3-Hydroxycinnamic acid	0.87	0.006	5.0
4-Hydroxycinnamic acid	1.27	0.009	5.0
α-Linolenic acid	7.73	0.053	2.0
γ-Linolenic acid	7.46	0.051	0.4
Chlorogenic acid	7.58	0.052	0.0
Caprylic acid	7.63	0.052	53.0
Decanoic acid	8.72	0.060	70.0
Erucic acid	7.18	0.049	0.0
Palmitoleic acid	7.94	0.054	1.0
Stearidonic acid	7.73	0.053	2.0

It is commonly known that Caco-2 monolayer is an excellent model of the passive transcellular pathway. Moreover, Caco-2 cell line is compared to the extensively folded human jejunum. However, there exists the hypothesis that only the villi tips comprising a fraction of the anatomical surface area of the intestine, participate in the absorption [44]. Among compounds studied, there exist three fatty acids which show high passive jejunum absorption: LUA, MA, CA and DA. These compounds are completely and fast absorbed in the human jejunum. The same substances also exhibited the highest Caco-2 permeability. On the contrary, the HCAs have the lowest jejunum permeability among compounds tested. This group of polyphenols is slowly and incompletely absorbed in the human jejunum. There also exist compounds which have high jejunum and simultaneously low Caco-2 permeability (order of magnitude: 10^{-7} cm s^{-1}): PA, LA, OA and GLA. Similar situation is observed in the case of substances which have high jejunum permeability and do not show any Caco-2 absorption: SA, CGA and EA. Such discrepancies between these two models may result from possible differences in the permeability of the paracellular pathway and in the absorptive surface areas. Compounds having a lower permeability will remain longer in the intestinal lumen before they are absorbed. Therefore, they may diffuse further down the length of the villi as compared to compounds which are rapidly and completely absorbed having thereby high permeability [8].

For all the compounds studied we constructed new models for describing the human jejunum and Caco-2 absorption using Abraham descriptors

$$P_{e,jejunum} = 0.000613 - 0.00189\mathbf{A} + 0.00158\mathbf{B} + 0.0019\mathbf{S} - 0.00193\mathbf{E} - 0.000152\mathbf{V}$$
$$\text{where } n = 17,\ S = 0.000035,\ \text{and } R^2 = 98.8\% \quad (9)$$

$$P_{e,Caco-2} = -0.000107 - 0.000282\mathbf{A} - 0.00006\mathbf{B} + 0.000808\mathbf{S} - 0.000514\mathbf{E} - 0.000066\mathbf{V}$$
$$\text{where } n = 17,\ S = 0.000011,\ R^2 = 81.8\% \quad (10)$$

On the basis of these two models, permeability through the human jejunum as well as Caco-2 monolayer of substances tested except for polyphenols (HCAs and CGA) has been estimated. Moreover, obtained values expressed in [cm s^{-1}] have been compared with those predicted *in silico* (see Table 5). The correlation between Caco-2 monolayer absorption predicted *in silico* and obtained from Eq. (10) has been achieved with R^2 equal to 0.81. Then, the correlation between human jejunum permeability estimated *in silico* and received using Eq. (9) was much better (R^2 nearly 0.99). This plot is presented in Fig. 5. As we can observe our new models are very suitable for intestinal permeability prediction of compounds studied.

3.5. Principal components analysis

There was an assumption that four substances (polyphenols) among all the compounds tested behave in the other way being slowly and incompletely absorbed in the human jejunum. In order to establish the relationships between different variables, the principal components analysis (PCA) has been done (Minitab 16 software). The results of PCA were obtained using steric, electronic, lipophilicity and physicochemical parameters as well as *in silico* jejunum, Caco-2 permeability and human intestinal % absorption parameters.

The first two principal components explain more than 95% of the variance in the data. Fig. 6 shows loading plot of $P_{e,jejunum}$, $P_{e,Caco\text{-}2}$, %Abs, log $P_{o/w}$ (and the other log $P_{o/w}$ parameters expressed as A log *P*s, AC log *P*, *x*log *P*2, M log *P*), molecular weight, molar refractivity, polarizability, parachor and molar volume. Values of $P_{e,jejunum}$, $P_{e,Caco\text{-}2}$, %Abs obtained using our new models are indicated in red. As we can see from the loading plot corresponding to the first two principal components, there is a high correlation between electronic and steric descriptors. However, %Abs and $P_{e,Caco\text{-}2}$ are the most distinctive from the rest of correlations because they are probably overvalued in relation to the other data.

In Fig. 7 score plot of $P_{e,jejunum}$, $P_{e,Caco\text{-}2}$, %Abs, log $P_{o/w}$, A log *P*s, AC log *P*, *x* log *P*2, M log *P*, molecular weight, molar refractivity, polarizability, parachor and molar volume are presented. Tested compounds have been divided depending on the structure into four different groups: saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids and polyphenols. It was important to confirm if polyphenols behave really differently than fatty acids.

We noticed that particular group behaves in the way typical of oneself, namely saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids with the exception of GLA which insignificantly diverges from its own group. By contrast, polyphenols behave completely different from fatty acids. In addition, their behavior is even different within group. It means that HCAs have very similar behavior but CGA significantly varies from them. These differences have been previously observed in obtained human intestinal absorption values. They can result mainly from their lipophilic as well as molecular structure properties such as topological polar surface area (TPSA) (see Table 4). Therefore, HCAs follow the Lipiński rule of five but CGA does not. These discrepancies determine the differences in human intestinal permeability behavior within polyphenols group.

4. Conclusions

In the present investigation saturated and unsaturated fatty acids as well as polyphenols have been tested. Their jejunum, Caco-2 and oral human absorption have been determined by means of *in silico* and *in vitro* measurements. Chromatographic data from Micellar Liquid Chromatography, particularly from Biopartitioning chromatography, were used for QRAR prediction of oral % absorption of compounds studied. Moreover, different chromatographic techniques using diversified surfactants (ionic

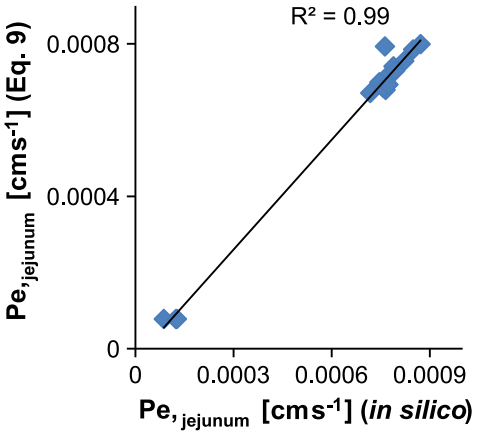

Fig. 5. The correlation between human jejunum permeability obtained on the basis of Eq. (9) and *in silico* predicted for chosen compounds tested.

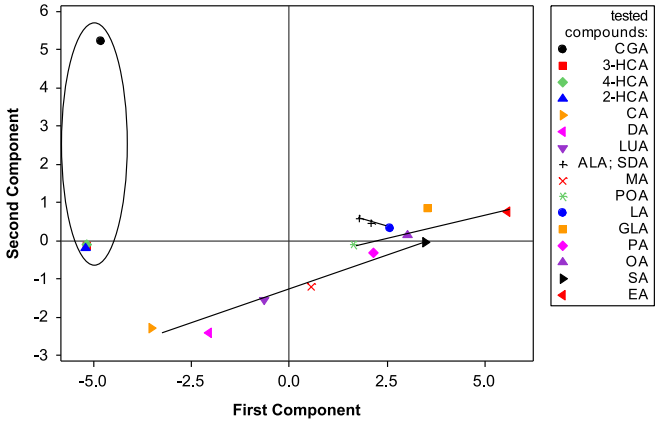

Fig. 7. Score plot of PC_2 against PC_1 for the principal component analysis of the compound descriptors according to the four groups of compounds: saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids and polyphenols.

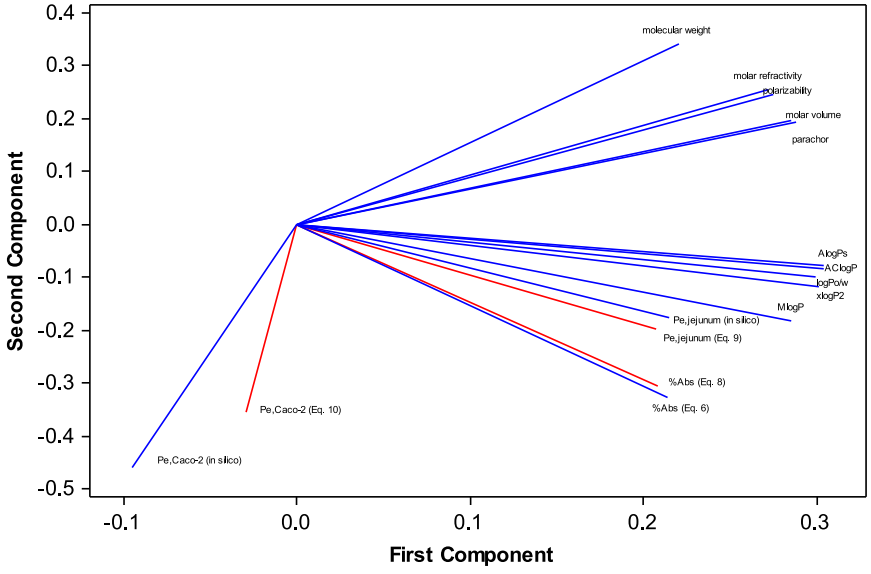

Fig. 6. Loading plot of $P_{e,jejunum}$, $P_{e,Caco\text{-}2}$, %Abs, log $P_{o/w}$, A log *P*s, AC log *P*, *x* log *P*2, M log *P*, molecular weight, molar refractivity, polarizability, parachor and molar volume. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article).

and non-ionic) in mobile phase have been compared. According to Foley's equation, retention factors extrapolated to pure water as well as analyte–micelle association constant have been received. On the basis of K_{ma} values possible interactions in different micellar environments have been identified.

However, the main purpose of this work was *in vitro* and *in silico* determination of human absorption of chosen fatty acids and polyphenols. The results of this study show that the retention of the tested substances obtained from BMC systems is capable to describe *in vitro* human oral absorption. The BMC is a very useful technique which can provide important information about the absorption properties. In addition, BMC is a more inexpensive way than cell culture or *in vivo* models being able to comprise screening method for absorption studies.

The completely new models of human absorption have been built to compare them with the cited models. Furthermore, many different correlations between physicochemical parameters and human intestinal absorption have been done. These very good correlations confirmed that our new constructed models are appropriate and very suitable for intestinal permeability prediction of saturated and unsaturated fatty acids as well as polyphenols.

By means of the computational method, Caco-2 and jejunum human absorption of the tested compounds have been determined *in silico*. Moreover, two different models for characterizing Caco-2 and jejunum absorption using Abraham parameters have been constructed. These *in silico* and newly constructed models have been compared to each other. The correlations between Caco-2 monolayer as well as jejunum absorption predicted *in silico* and obtained from our two models were satisfactory (R^2 0.81 and 0.99, respectively).

According to miscellaneous tested models four compounds behave in a different way in relation to other compounds. These substances belong to polyphenols (2-HCA, 3-HCA, 4-HCA, CGA) and can be classified as strong Brønsted acids. Therefore, they are distinguished by different human absorption behaviors in contrast to fatty acids. To confirm that, the PCA has been done.

The presented work approach can be very useful in pharmaceutical and medical chemistry research. Since the tested compounds can be components of different bioactive plant extracts (*e.g.* of raspberry seeds (*Rubus idaeus*), strawberry seeds (*Fragaria ananassa*), blackcurrant seeds (*Ribes nigrum*), aronia seeds (*Aronia Medik.*), Japanese rose seeds (*Rosa rugosa Thunb.*) and palmetto palm fruit (*Sabal minor*)), the presented approach may be utilized *e.g.* for quality evaluation of raw materials in a commercial production of wide range of many different goods.

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