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Comparison of micellar chromatography systems with buffer and simulated body fluid solutions as component of mobile phases

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Abstract: The study evaluated two micellar biomimetic chromatographic systems for predicting the biological activity of compounds. Drugs with various structures and pharmacological profile were used as test substances. Two chromatographic systems were tested with a C-18 stationary phase and mobile phases: *n*-propanol and micellar simulated body fluid (SBF) solutions or buffer with a physiological *pH* of 7.4 at different concentrations of the surfactant SDS. Linear relationships were observed for the retention coefficients *k= f(C), log k= f(C)* and *1/k= f(C)* (C- surfactant concentration). From these relationships, the values of k_0, *km* and K_{AM} were determined and their relationships were examined. The analyzed data showed that the composition of the aqueous mobile phase solution affects interactions within the micellar system. Comparison the obtained values with selected descriptors of biological activity of substances, revealed strong correlations between log K_{AM} values (analogue-micelle association constants) and log K_A *(HSA)* values enabling the prediction of drug binding to proteins based on MLC measurements. In addition, K_{AM} values inform us about potential for drug deposition in micellar systems.

Keywords: micellar liquid chromatography, *SBF* and buffer as components of mobile phases, descriptors of biological activity.

1. Introduction

Chromatography – apart from being an invaluable method of qualitative and quantitative analysis and a method of obtaining pure substances on a preparative scale, it is also a method of great importance in research on the biological activity of compounds (Hostettman et al., 1998; Bai et al.; 2021, Primdahl et al., 2022: Hussein, 2025;). Chromatography is becoming increasingly important in research on the biological activity of compounds (Grządka et al., 2024), through studies of compound retention in chromatographic systems that mimic their environment of action in organisms – in so-called biomimetic chromatographic systems (Ulenberg et al., 2022; Valko, 2022; Tsopelas et al., 2025.). The use of biomimetic chromatographic systems is aimed at determining the interaction of biologically active substances with:

- cell membranes – through biomimetic chromatographic systems, i.e. systems in which the stationary phases imitate biological membranes, such as:
- *RP* (reversed phase) chromatography with *C-8* stationary phases, *C-18* – imitating the interior of the cell membrane, allowing the determination of the possibility of substance penetration through the lipophilic area of the cell membrane (Janicka et al., 2006, Poole et al., 2020, Poole et.al., 2024).
- *RP* chromatography with stationary phases immobilised with cell membrane components, i.e.: IAM phases (Pidgeon, Venkataram, 1989; Ermondi et al., 2018, Russo et al, 2021, Wang et al., 2024, Zhu et al., 2022), cholesterol phases (Grzywiński et el., 2015; Pesek et al., 2022; Pesek et al., 2023),

Micellar liquid chromatography (*MLC*), in which both the RP stationary phase and the micelles in the mobile phase imitate cell membranes (Quinones-Torrelo et al., 2002; Rambla-Algere et al., 2012; Shokry et al., 2018), affinity chromatography (Hage, 2002; Matsuda et al., 2015; Hage, 2017), including

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its special case of cell membrane chromatography, which can be used to determine the interactions of substances with membrane receptors (Du et al., 2011, Bu et al., 2022, Lv et al., 2017, Ma et al., 2017).

Also based on chromatographic studies, *QRAR* (quantitative retention - activity relationships) models can be used to predict descriptors of the biological activity of substances (Valko et al., 2001; Quinones-Torrelo 2002; Valko et al., 2003; Wang et al., 2007; Ciura et al., 2020; Ulenberg et al., 2022).

Micellar liquid chromatography, pioneered by Armstrong (Armstrong et al., 1981, Armstrong 1985) is an interesting variation of chromatography in which the stationary phase is the *RP* phase – most often phases with alkyl ligands, less often *RP-NH*$_2$ (amino) or *RP-CN (*cyano*)*, and the mobile phase is an aqueous solution of surfactant with a concentration above critical micellar concentration - *CMC*. In such a system, the retention of substances depends on their interaction of the substances with both the stationary phase and the mobile phase micelles (Fig. 1) – this mechanism is a more complex mechanism than that in classical *RP* chromatography.

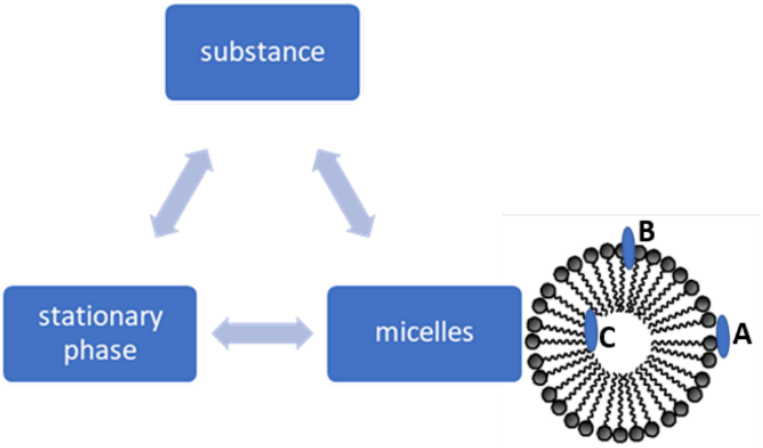


Fig. 1. Interaction in micellar chromatographic system, A-lipophobic substance, B – weak lipophilic substance, C-strong lipophilic substance

MLC systems in which the mobile phases are aqueous surfactant solutions usually have low efficiency due to the low transfer rate of substances between the stationary phase and micelles. The efficiency of such systems can be improved by increasing the measurement temperature or by adding low-molecular-weight aliphatic alcohols (from *C-1* to *C-5*) or organic modifiers used in classical *RP* chromatography. Such additives usually reduce the retention of the tested substances and improve the efficiency of the chromatographic system. The concentration of an organic modifier must not be too large, as this may cause micelle disintegration (Giddings, 1987; Tsopelas et al., 2020; Valko, 2022).

The retention of substances depends on the concentration of micelles in the chromatographic system. Depending on how the retention of substances changes with the change in surfactant concentration, substances are classified into:

- binding – if their retention decreases with increasing surfactant concentration in the mobile phase, indicating that the tested substances interact with the micelles,
- non-binding – if retention does not depend on surfactant concentration, indicating that the substances do not interact with the surfactant micelles,
- anti-binding – if the retention of the substance increases with increasing surfactant concentration, indicating that the substance is repelled by the micelles and interacts more strongly with the stationary phase. Such analytes are usually encountered when the surface charge of the micelles and the tested substance are the same.

The relationship between substance retention and micellised surfactant concentration is described by Foley's equation (Foley, 1990):

$$\frac{1}{k} = \frac{1}{k_0} + \frac{K_{AM}}{k_0}[M] \quad (1)$$

where [M] - concentration of the micellised surfactant, k - retention factor of the substance in the mobile phase with the surfactant concentration [M], k_0 - retention factor of the substance at *Ccmc* concentration of the surfactant, K_{AM} – solute- micelle association constant.

The mathematical form of Foley's equation suggests that the inverse of the retention coefficient of a substance is a linear function of the concentration of micelles in the mobile phase. By determining the parameters of the linear relationship of Foley's equation, we obtain the values of the parameters k_0 and K_{AM}, provide information about the interactions of the tested substance with both the stationary phase and the micelles. This equation has been tested experimentally for many substances (natural, ionic – cations and anions, polar and non-polar) as well as surfactants (anionic, cationic, non-ionic) and stationary phases (*C-8, C-18, RP-CN*).

Micellar chromatography is highly significant as a method in clinical analyses due to the possibility of solubilising proteins in micelles, greatly simplifying the preparation of biological samples for analysis (Sharma et al., 2022; Leelakunakorn at el., 2023). It is also of great importance as a method for determining the biological activity descriptors of compounds (Breyer et al., 1991; Waters et al., 2007; Sobanska et al.,2019; Tsopelas et al., 2020; Sharma et al., 2022; Krongvorakul et al., 2023).

To accurately reflect the environment in which the drug acts, in addition to stationary phases that imitate cell membranes or the site of interaction between the drug and the receptor, the mobile phase is also crucial in reproducing the environment in which the drug acts. In all the chromatographic methods mentioned, the mobile phases are aqueous solutions – either electrolytes or aqueous-organic solutions. Research on the biological activity of compounds is most often conducted in mobile phases at the pH at which the drug acts. This is usually the physiological pH of blood plasma – 7.4, as every drug is transported by the blood to its site of action. Another, more advanced biomimetic mobile phase consists of a solution simulating biological fluids – *SBF* (Pietrzyńska et al., 2017, Shokry at el., 2018). The aim is to ensure that biomimetic systems replicate the drug's operating environment as closely as possible. In the case of chromatographic systems, this applies to both the stationary and mobile phases. The physiological *pH* of the mobile phase allows the study of interaction between substances (ionised or not, depending on their properties) and stationary phases that imitate biological membranes or their fragments. Since substances in the body must migrate across the lipophilic regions of biological barriers, the form in which the substance exist is of great importance. The biological activity of a substance depends on the interfacial phenomena, which are determined by the composition of physiological fluids. Therefore, it is reasonable to investigate whether and how the interactions of biologically active substances with surfaces imitating biological barriers depend not only on *pH* but also on the electrolyte composition of the "aqueous" part of the mobile phase. Thus, it was decided to compare micellar chromatographic systems with hybrid mobile phases: micellar *SDS* buffer solutions with a physiological *pH* of 7.4 and micellar phases of solutions simulating the composition of physiological fluid (*SBF*).

2. Materials and methods

2.1. Investigated substances

Investigated substances are presented in Table 1.

Initial solutions of the tested drugs were prepared at concentrations of 1 mg·cm^{-3}, using methanol (for *HPLC*, Merck, Germany) as the solvent. Dilutions with appropriately selected concentrations were made from the resulting samples based on the analysis of chromatograms of concentrated drug samples. In order to obtain quantitative relationships between the concentration of the analysed substance and the area under the peak. Five concentrations were prepared for each substance. The list of concentrations for each substance is shown in Table 2.

2.2. Chromatographic measurements

The measurements were performed using the micellar liquid chromatography (*MLC*) method with a Shimadzu Vp liquid chromatograph equipped with:

- *LC 10AT* pump,
- *SPD 10A UV-VIS* detector,
- *SCL 10A* system controller,
- *CTO-10 AS* oven,
- 20µL dosing loop valve (Rheodyne, Cotati, *USA*).

Table 1. Name and chemical formula of investigated substances

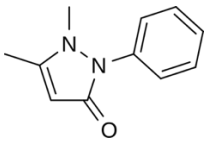
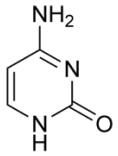
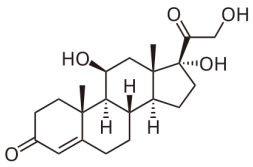
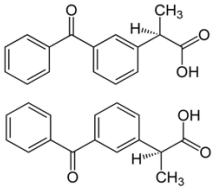
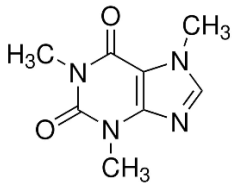
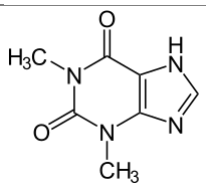
Name of substances	Semi-structural formula
antipiryne	
cytosine	
hydrocortisone	
ketoprofen	
caffeine	
theophilline	

Table 2. Concentrations of the analyzed substances used for calibration curves

substance	concentration [μg·mL^{-1}]				
	1	2	3	4	5
antipirine	0.2	0.3	0.4	0.5	0.6
cytosine	0.3	0.4	0.5	0.6	0.7
hydrocortisone	0.3	0.4	0.5	0.6	0.7
ketoprofen	0.2	0.3	0.4	0.5	0.6
caffeine	0.5	0.6	0.7	0.8	0.9
theophylline	0.4	0.5	0.6	0.7	0.8

During the measurements, a mobile phase flow rate of 0.5 cm^3·min^{-1} was used. The analyses were performed at a column operating temperature of 20^{0}C. The detection of the tested substances took place at a wavelength of λ=254 nm.

The stationary phase was a sorbent bed located in an *RP-18 HPLC* column from Merck (Germany, Darmstadt).

In the experimental part of this work, two series of micellar mobile phases (*S* and *B* systems) with the following composition were used:

- *S*: organic modifier *n*-propanol (20% v/v), aqueous phase – *SBF* (Simulated Body Fluid) solution, *SDS* surfactant (sodium dodecyl sulphate) from Merck (Germany),
- *B*: organic modifier *n*-propanol (20% v/v), aqueous phase – citric buffer (pH=7.4), *SDS* surfactant.

For each system, four mobile phases were prepared, in which the surfactant concentration was: 0,04, 0.06, 0.08, and 0.10 M dm^{-3}.

2.3. Preparation of mobile phases

2.3.1. Preparation of the *SBF* solution

An aqueous solution of *SBF* (Simulated Body Fluid) with a *pH* of approximately 7.4 was prepared from the following chemical compounds: 7.996 g NaCl, 0.350 g $NaHCO_3$, 0.224 g KCl, 0.228 g $K_2HPO_4 \cdot 3H_2O$, 0.305 g $MgCl_2 \cdot 6H_2O$, 40 cm^3 1M HCl, 0.278 g $CaCl_2$, 0.071 g Na_2SO_4, 6.057 g $(CH_2OH)_3CNH_2$. An important factor during the preparation of *SBF* is the order in which the above-mentioned substances are added. Then, by adding small portions (0.5-1.0 mL) of hydrochloric acid, the *pH* of the solution was adjusted to approximately 7.4 using a pH meter.

2.3.2. Preparation of citric buffer

In order to prepare a citric buffer with a pH of approximately 7.4, aqueous solutions were prepared: disodium hydrogen orthophosphate (V) (Merck, Germany) at a concentration of 7.16 $g \cdot dm^{-3}$ and citric acid (Merck, Germany) at a concentration of 2.1 $g \cdot dm^{-3}$, and mixed them together in a ratio of 10:1 (900 cm^3 Na_2HPO_4 and 90 cm^3 citric acid). Then, using a pH meter, the *pH* of the prepared buffer was measured, which was approximately 7.4. Milli-Q distilled water from a Millipore apparatus (Simplicity, Millipore, USA) – conductivity 0.0055 µS cm^{-1}, resistance 18.2 MΩ cm^{-1}, was used to prepare all of the above-mentioned solutions.

3. Results and discussion

3.1. Surface areas of chromatographed substances in micellar mobile phases *S* and *B*

Since micellar chromatography can be used for quantitative studies, the effect of the mobile phase type on the detector signal and calibration curve parameters was investigated. Fig. 2 shows the calibration

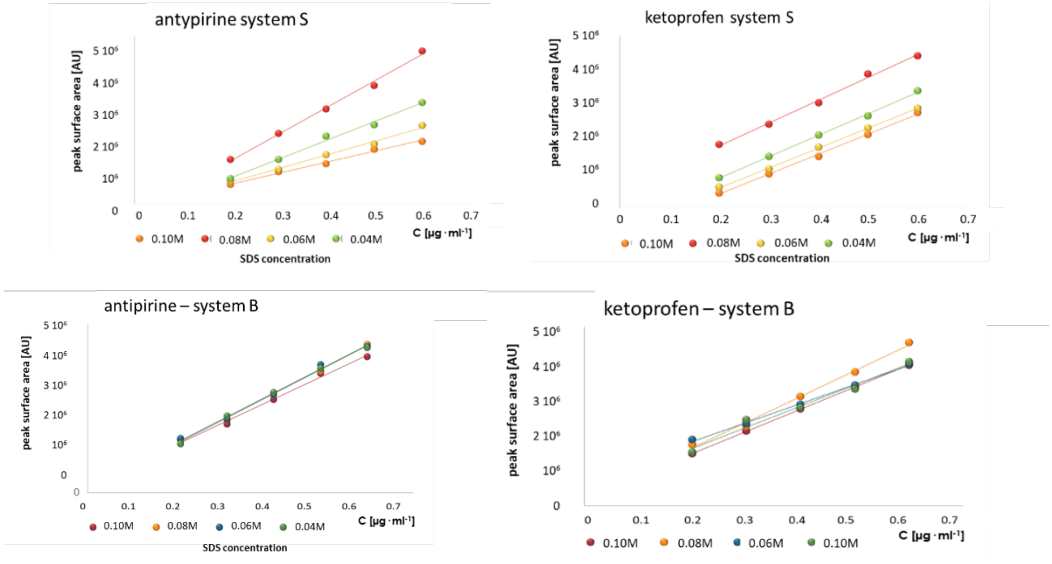


Fig. 2. Calibration curves obtained in investigated micellar system (*S* and *B*) in mobile phases with different concentration of *SDS*

curves for selected substances: antipyrine and ketoprofen obtained in systems *S* and *B* at different *SDS* concentrations.

As shown by from the attached data, in systems with an *S* mobile phase, the calibration curves parameters strongly depend on the surfactant concentration in the mobile phase. The tested substances can be divided into two groups. The first group includes: antipyrine, cytosine, caffeine, and theophylline, for which a change in the surfactant concentration alters in the sensitivity of the method (i.e. the slope *a* of the calibration curve) - as the surfactant concentration decreases, the sensitivity of the method decreases. For the remaining substances (hydrocortisone and ketoprofen), the surfactant concentration has virtually no effect on the sensitivity, but it does affect the limit of detection (*LOD)* and limit of quantification (*LOQ)* values (Table 3). For this reason, calibration curves for two selected substances – one from each group – antipyrine and ketoprofen – are presented graphically. In systems with a buffer as a component of the mobile phase, these relationships are less apparent.

Table 3. Parameters and R^2 of calibration curves of substances obtained with micellar system with *S* and *B* mobile phase

substance		*S mobile phase*			*B mobile phase*		
	SDS conc.	*a*	*b*	R^2	*a*	*b*	R^2
antypirine	0.10	$3 \cdot 10^6$	-41117	0.9922	129497	199788	0.9921
	0.08	$8 \cdot 10^6$	-280838	0.9951	141733	104468	0.9974
	0.06	80333	-128713	0.9933	141977	128109	0.9940
	0.04	111888	-318715	0.9918	142657	84660	0.,9977
cytosine	0.10	$1 \cdot 10^6$	-156141	0.9983	77058	962614	0.4210
	0.08	$3 \cdot 10^6$	118920	0.9304	83028	$1 \cdot 10^6$	0.,6564
	0.06	27429	-180293	0.9852	68161	2000000	0.3485
	0.04	108268	$-1 \cdot 10^6$	0.9336	77181	2000000	0.3564
hydrocortison	0.10	$2 \cdot 10^6$	497532	0.9913	37312	$1 \cdot 10^6$	0.9462
	0.08	$5 \cdot 10^6$	503291	0.9944	57791	$1 \cdot 10^6$	0.9888
	0.06	78263	216255	0.9994	58544	$1 \cdot 10^6$	0.9701
	0.04	74111	$1 \cdot 10^6$	0.9954	65631	587145	0.8926
ketoprofen	0.10	$6 \cdot 10^6$	-755208	0.9974	124162	219318	1.0000
	0.08	$7 \cdot 10^6$	486934	0.9952	144340	171594	0.9954
	0.06	115406	-563765	0.9995	106475	733145	0.9981
	0.04	124778	-377807	0.9981	117930	427292	0.9797
caffeine	0.10	776078	-136019	0.9969	25815	719543	0.9650
	0.08	$2 \cdot 10^6$	45808	0.9886	38413	459052	0.9661
	0.06	36063	239791	0.9421	35184	624188	0.9987
	0.04	27987	498431	0.9981	36832	498301	0.9847
theophylline	0.10	429234	55018	0.9863	54798	707553	0.9837
	0.08	$2 \cdot 10^6$	598701	0.9854	66647	402239	0.9916
	0.06	57817	268051	0.9977	60416	769445	0.9534
	0.04	32993	676120	0.9882	68396	408429	0.9938

3.2. Study of the relationship between retention data in *MLC* systems with *S* and *B* mobile phases

Since differences in the retention of the tested substances were observed in the analogous systems differing in the composition of the aqueous part of the mobile phase, the relationships between these values were also investigated. For all tested substances, linear relationships were observed between the retention coefficients of the tested substances with high correlation coefficients (Fig. 3). As the surfactant concentration increases, the differences in retention for all tested substances decrease (Fig. 4).

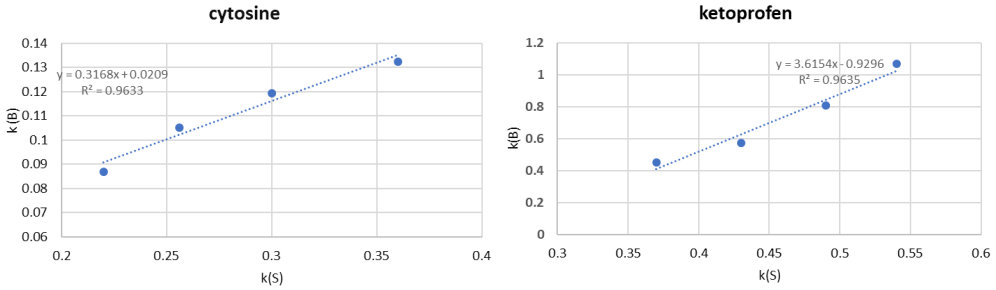


Fig. 3. Examples linear relationship between retention factor values for substances obtained in micellar system with *S* (*k(S)*) and *B* (*k(B)*) mobile phases with different concentration of *SDS*

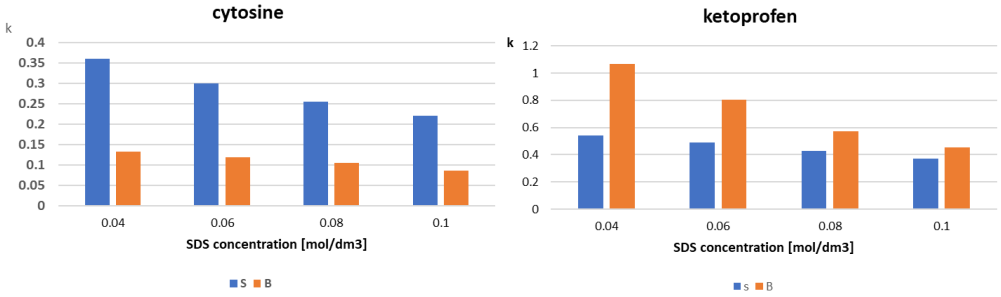


Fig. 4. Comparison of the retention coefficients *k* of substances obtained in *S* and *B* mobile phases with different surfactant concentrations

3.3. Relationship between substance retention coefficients and surfactant concentration in MLC systems

The relationships between retention values and the concentration of micellised surfactant in the mobile phase were investigated. For this purpose, the relationships between retention values and the concentration of micellised surfactant were investigated, i.e.:

- log k = f(C), which occur in *RP* systems,
- k= f(C),
- Foley's dependence 1/k=f(C).

All these dependencies are presented for individual substances in Fig. 5.

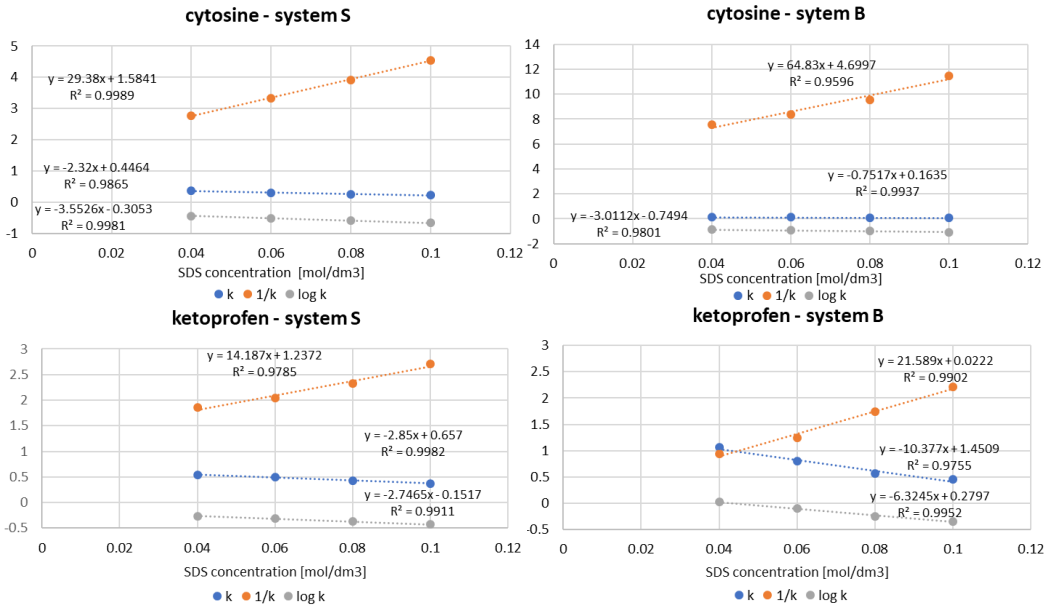


Fig. 5. Example relationship between retention factor *k*, log *k*, 1/*k* and concentration of micellised *SDS* in mobile phase

As shown by the data presented in the graphs, all the relationships studied are linear with high coefficients of determination. As the systems examined are classified as biomolecular systems, the parameters of linear relationships are of great importance.

For the linear relationships log k = f (C) and k = f (C), the slope of may indicate the hydrophobicity of the substance demonstrating - how changes of compositions of mobile phase affect in the lipophilic properties of substance, while the free term of the relationship indicates the value of the substance's retention coefficient in a system without micelles, i.e. the value of the substance retention coefficient in a system with a mobile phase at the *CMC* concentration of the surfactant (k_m). Table 4 compares the slopes of the linear relationships k= f (C)

Table 4. Comparison of absolute values of slope of linear relationships k= f (C) in *MLC* systems with *S* and *B* mobile phases

substance/	S	*B*
antipirine	1.65	1.65
cytosine	2.32	0.7517
hydrocortisone	5.50	13.09
ketoprofen	2.85	10.38
caffeine	3.25	0.75
theophylline	2.57	0.37

The slope of the linear relationship does not change for antipyrine (consistent with similar its retention in the two tested systems), for hydrocortisone and cytosine it is greater in the system with a buffered mobile phase than in *SBF*, while for the remaining compounds it decreases in the mobile phase with a buffer compared to the *SBF* mobile phase. The values of retention coefficients k_m obtained on the basis of the linear dependencies log k = f(C) and k = f(C) in the tested chromatographic systems were compared. Regardless of the determination method, linear dependencies with a high fit coefficient were obtained in both tested systems (Fig. 6).

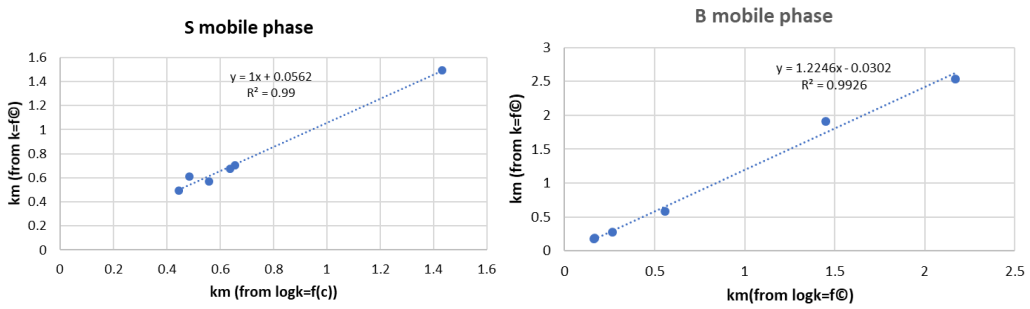


Fig. 6. Relationships between k_m parameter obtained from k= f (C) and log k = f (C) relationships

However, there are no such strong relationships between the k_m values obtained in micellar systems according to Foley equation with the S and B mobile phases. The two substances, ketoprofen and theophylline, do not correlate with each other in either system – ketoprofen is ionised at pH=7.4, while theophylline is partially ionised in this environment (Fig. 7).

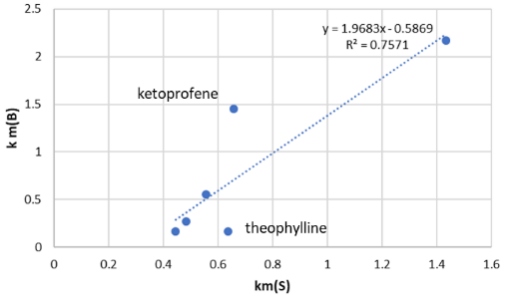


Fig. 7 Relationship between *km* values obtained in micellar systems with *S* and *B* mobile phase

As shown by the data in the table, the K_{AM} values are higher than the k_0 values, which further confirms the strong binding nature of the tested compounds in relation to micelles. It can also be observed that both k_0 and K_{AM} values are higher in systems with *SBF* as the mobile phase than in systems with buffer. This is probably due to the greater ionic strength of the *SBF* system compared of the buffer and the action of chaotropic ions in *SBF* solutions (Flieger et al., 2008; Vemić et al., 2014; Cecchi 2024). Such studies may be useful for designing new micellar systems for drugs incorporation, which can then be administered to patients (Kapare et al., 2020; Qiu et al., 2021; Wang et al., 2023). The correlations between the obtained parameters from the considered linear relationships were examined, and – the results are presented in the correlation matrix (Table 6).

Table 6 Correlation matrix between parameters determined from linear relationships k= f(C) and $1/k$ = f(C) in investigated systems

	$k_m(S)$	$k_m(B)$	$K_{AM}(S)$	$k_0(S)$	$K_{AM}(B)$	$k_0(B)$
$k_0(S)$	1.000					
$k_0(B)$	0.970	1.000				
$K_{AM}(S)$	-0.332	-0.252	1.000			
$k_m(S)$	-0.009	-0.197	-0.049	1.000		
$K_{AM}(B)$	-0.310	-0.230	-0.053	-0.462	1.000	
$k_m(B)$	0.332	0.329	-0.378	-0.450	-0.350	1.000

The values of the k_0 parameters obtained from the linear relationships between retention and surfactant concentration in *S* systems are orthogonal to the k_m values obtained from Foley's equation, while a weak positive correlation is observed with the km values obtained in the buffer system. When comparing the correlations between the K_{AM} values obtained in buffer and SBF systems, we also observe an orthogonal relationship, which may indicate a change in the interactions of substances with micelles in chromatographic systems with the considered mobile phases. The sorption of substances from electrolyte solutions is a complex and not entirely predictable process. Although the Hofmeister effects play an important role in many biological processes and phenomena, influencing the zeta potential, enzyme activity, and the binding of substances to micelles, proteins, and membranes, they are not yet fully understood (Vlachy at al., 2008). Since the obtained parameters are often used to determine the lipophilicity of substances, it was decided to examine the correlations between these values and the values of selected biological activity descriptors, i.e. log *P* (Meylan-Kowwin model assessment), *M*log*P* and *A*log *P* – models were selected whose values are not too highly correlated (Table 7). Due to the micellar nature of the mobile phases, correlations between the obtained parameters and the logarithm of the binding constant of the tested drugs with *HSA* (log K_a *(HSA)*) as well as correlations with the solubility of the tested substances in water were also investigated. The correlations between the tested descriptors of biological activity of the substances are presented in Table 8.

Table 7. Correlation matrix between selected descriptors of biological activity of substances (a-model (Meylan-Kowwin)-assessment, b-model (*M*Log*P*)-prediction, c-model (*A*Log*P*)-prediction)

	logP^a	*M*logP^b	*A*logP^c	log $K_{a(HSA)}$	solubility
logP^a	1.000				
*M*logP^b	0.738	1.000			
*A*logP^c	0.782	0.782	1.000		
log $K_{a(HSA)}$	0.061	0.019	0.165	1.000	
solubility	-0.370	-0.530	-0.459	-0.344	1.000

When analysing the correlations presented in Table 8, strong negative correlations were observed between the log k_0 (*S*) value and the log *P* values obtained according to different models, as well as a strong positive correlation between the log k_0 *(B)* values and the log *P* values. Also noteworthy are the

negative correlations between log Ka(*HSA*) and the parameter log $k_0(S)$, which are - medium and strong between log $K_{AM}(S)$, log $K_{AM}(B)$, and the strong positive correlation between the log k_0(B) values.

Table 8. Correlation matrix between the log of the parameters obtained from the linear relationships between the retention coefficient of the substance and its concentration and the log of the values of selected descriptors of the biological activity of the tested compounds

	$logk_{m(S)}$	$logk_{m(B)}$	$logK_{AM(S)}$	$logk_{0(S)}$	$logK_{AM(B)}$	$logk_{0(B)}$
logP [a]	-0.283	0.138	-0.345	-0.730	0.606	-0.092
*M*log*P* [b]	-0.613	-0.009	0.072	-0.787	0.708	0.035
*A*Log*P* [c]	-0.042	0.296	-0.366	-0.721	0.699	0.272
log *Ka*(*HSA*)	0.269	0.515	-0.614	-0.482	-0.584	0.958
solubility [mg/L]	-0.024	-0.760	-0.188	0.847	-0,471	-0.381

4. Conclusions

Two biomimetic micellar chromatographic systems differing in the composition of the 'aqueous' part of the mobile phase were tested: one – with an SBF solution simulating the composition of blood plasma, and one with a buffer at *pH* equal to that of blood plasma (*pH*= 7.4. Drugs with different structures and pharmacological activities were used as test substances. It was shown that the electrolyte composition of the hybrid micellar mobile phase affects the retention of the test substances and that there are strong linear correlations between the retention values for given substances in analogous mobile phases. The relationships between retention values and surfactant concentration in the mobile phase were also investigated. For the two systems tested, linear relationships with high coefficients of determination were observed for all tested substances between: the retention coefficient, logarithm of the retention coefficient, and the inverse of the retention coefficient of the substance and the concentration of the micellised surfactant. It was shown that there is practically no correlation between the obtained parameters of linear relationships in the tested systems. However, correlations were observed between the logarithm of the parameters obtained from the Foley equation and the logarithm of selected descriptors of the biological activity of the substance, indicating that the tested systems can be treated as biomimetic systems. Particularly high correlations were observed between the parameters of the Foley equation and the log *Ka(HSA)* values, suggesting that the tested systems can be used to predict the degree of the degree of drug binding to protein. Analysis of K_{MA} parameter values in the tested systems indicated that the substances bind more strongly to micelles in systems with *SBF* then buffer solution as components of the mobile phase.

Although the presented studies are pilot studies, they demonstrate that the electrolyte composition of micellar solutions used as biomimetic systems for predicting the biological activity of compounds is meaningful, as it reveals changes in the interactions of the tested substances with micelles and the stationary phase compared to analogous systems with buffer solutions. The use of chromatographic micellar systems as biomimetic systems.

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