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## INFLUENCE OF THE STRUCTURE OF BILE ACIDS ON THEIR PARTITION COEFFICIENT IN DIBUTYL ETHER AND CHLOROFORM

**ABSTRACT:** Bile acids are well known natural surfactants able to modify the permeability of biological membranes. The logarithm of partition coefficient between, traditionally used, *n*-octanol and water is a measure of lipophilicity as a predictor of solute membrane partitioning.

The aim of this work was to determine partition coefficients of bile acids in a mixture of water and chloroform and dibutyl ether at different pH values and with addition of different concentrations of sodium ions, and to examine the influence of the structure of bile acid nucleus on measured partition coefficients.

Partition coefficients of three bile acid salts were determined using shake-flask method and the concentration of bile acids was determined after twelve hours of shaking at the room temperature in aqueous and organic layer using reversed phase HPLC with DAD detector on 210 nm.

For all three analysed bile acid salts values of logP are lower in dibutyl ether than in chloroform. At certain pH values, curves representing the dependence of partition coefficient on pH value intersect, and these are the pH values for which partition coefficients are the same for both solvents. Increasing the solution ionic strength, this intersection is shifted toward lower pH values. It is found that, for both organic solvents, after the addition of hydroxyl group in the steroid nucleus (i.e. if the bile acid is less hydrophobic) the value of logP falls, especially if more hydroxyl groups are present. With chloroform as a solvent, system quickly comes to excess with electrolyte ions than with dibutyl ether.

**KEYWORDS:** bile acids, chloroform, dibutyl ether, partition coefficient

### INTRODUCTION

Bile acid salts (BA) are amphiphilic steroids with two functionally different molecular surfaces, convex area which is more hydrophobic, and concave surface

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of the steroid core, a less hydrophobic one. BAs are characterized by different number, position and orientation of OH/oxo groups in different C atoms of the steroid skeleton with a consequent influence on their physico-chemical and biological properties [Poša 2011].

In addition to facilitating lipid digestion and absorption, bile acids are critical regulators of many key aspects of intestinal function – cell growth and death, epithelial barrier and transport function, mucus secretion, mucosal immune function, and intestinal motility [Keating 2009]. All these led to a growing interest in their analogues in metabolic disorder therapeutics [Mikov 2007]

Beside that, BAs are used as promoters in transport of some drugs [Mikov 2007; Gordon 1985; Bove 1997; Poša 2008], and they have been extensively studied as permeability enhancers of various membranes [Yang 2009].

It is shown that electrolyte presence has an important influence on changes in physico-chemical features of bile acids as well as on their modular properties [Yang 2009; Roda 1983].

The logarithm of partition coefficient between *n*-octanol and water ( $\text{Log } P_{oct}$ ) is traditionally used measure of lipophilicity as a predictor of solute membrane partitioning. In many situations,  $\text{Log } P_{oct}$  cannot give a good estimate of the absorption of a drug or its permeation [Roberts 1996; Roberts 1996; Pugh 1996; Raevsky 2000; Wohnsland 2001]. Thus, other solvent systems are needed to yield information that is complementary to  $\text{Log } P_{oct}$  data [Okada 1985]. Four classes of solvent/water systems are suggested to model the partitioning of solutes into membranes [Okada 1985; Leahy 1992a and 1992b]: an amphiprotic solvent such as octanol, a H-bond acceptor solvent such as dibutyl ether, a H-bond donor solvent such as chloroform, and an aprotic inert solvent such as alkane or 1, 2-dichloroethane.

The objective of the investigation reported here was to determine partition coefficients of three bile acids salts (sodium cholate (NaC), sodium deoxycholate (NaD), and sodium lithocholate (NaL) (Fig. 1)) between water and chloroform, and dibutyl ether and water at different pH values and with addition of different concentrations of ions (sodium ions), and to examine the influence of the structure of bile acid nucleus on measured partition coefficients.

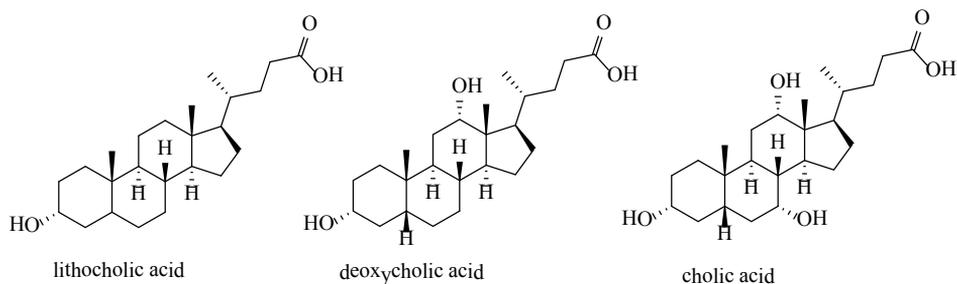


Figure 1.

## MATERIALS AND METHODS

Cholic, deoxycholic and lithocholic acid (Sigma, New Zealand, 98%) were transformed to their sodium salts by a known procedure [Roda 1983]. Methanol, HPLC grade, was obtained from Carlo Erba reagenti, Italy, and  $\text{KH}_2\text{PO}_4$  and  $\text{Na}_2\text{PO}_4$  from Lachner, Czech Republic. Chloroform (HPLC grade) was purchased from Alfa Aesar and NaCl pro analysis from Merck, Germany.

Partition coefficients were determined using shake-flask method. Solutions of bile acids (NaC, NaD and NaL) were prepared in 0.01 M phosphate buffers at different pH values (pH 5.8, pH 6.2, pH 6.6, pH 7, pH 7.4, pH 7.8, pH 8), of different ionic strength adjusted with sodium chloride (0, 0.05M NaCl, 0.1M NaCl, 0.15M NaCl and 0.2M NaCl), and mixed with the same volume of non-polar phases chloroform and dibutyl ether respectively. Concentration of each bile acid was 4mmol/L in the whole volume. Solutions were mixed for 12 hours using magnetic stirrer. Concentrations of bile acids were determined in both phases using validated HPLC method.

The HPLC system Agilent 1100 Series, equipped with degasser, binary pump, automatic injector and DAD detector with software system for data processing AgilentChemStation was used and the analyses were performed on a reversed-phase C-18 column: Eclipse Plus C18 (250 mm x 3 mm, 5  $\mu\text{m}$ , 250  $\text{Å}$ ) column (Zorbax SD). The mobile phase was 0.01M phosphate buffer: methanol = 70: 130 v/v maintained at pH 7 and the injection loop was 10  $\mu\text{L}$ . All separations were performed isocratically at a flow rate of 1 ml/min and a column temperature changing of 20. The detection was performed at 210 nm [Roda 1990].

## RESULTS AND DISCUSSION

It is important to know partition coefficients (of ionised and unionised form) of bile acids in different organic solvents, since certain types of solution more or less model some organs or anatomic units in biological systems. For example, dibutyl ether models very well the transferring of substances over blood brain barrier.

For all three analysed bile acid salts values of  $\log P$  are lower in dibutyl ether than in chloroform. There are no data on bile acid partition coefficients in chloroform and dibutyl ether. Literature data on  $\log P$  values of cholic acid and deoxycholic acid in 1-octanol are higher than in chloroform ( $\log P_{\text{oct}} > \log P_{\text{chloroform}} > \log P_{\text{dibutyl ether}}$ ) [Roda 1990]. We suggest that the highest values of partition coefficient of  $\log P_{\text{oct}}$  resulted from the fact that 1-octanol have OH group which is proton acceptor and proton donor at the same time so it has higher ability to form hydrogen bond with OH groups of the  $\alpha$  side of the steroid skeleton than in case of chloroform which is proton donor and dibutyl ether, a proton acceptor (Fig. 2).

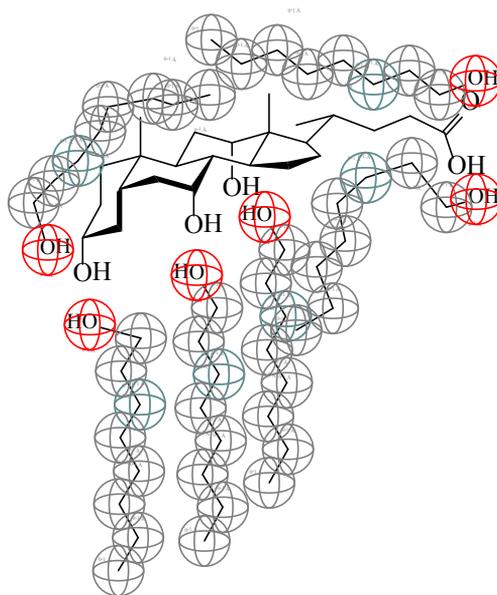


Figure 2.

Also, geometry of 1-octanol molecule makes favourable conditions for solvation of steroid skeleton comparing to chloroform and dibutyl ether. Namely, in 1-octanol polar OH group and hydrophobic tail are completely separated, and if we take into account a high conformational flexibility of octile tail during building of solvation shell, its translation entropy changes a bit compared to chloroform and dibutyl ether (1-octanol participates less in solvation of steroid skeleton than chloroform and dibutyl ether, which is a result of molecular flexibility of 1-octanol which adapt to geometrical shape of the steroid skeleton). The lowest value of partition coefficient for dibutyl ether is probably because two hydrophobic units of this molecule are separated with oxygen bridge which lowers hydrophobic solvation of this molecule.

At certain pH values, curves representing the dependence of partition coefficient on a pH value intersect. These are the pH values for which partition coefficients are the same for both solvents. Increasing the solution ionic strength, this intersection is shifted toward lower pH values (Fig. 3). Solvation effect is more prominent if there is a higher fraction of unionised form of the molecule in equilibrium system (at lower pH values).

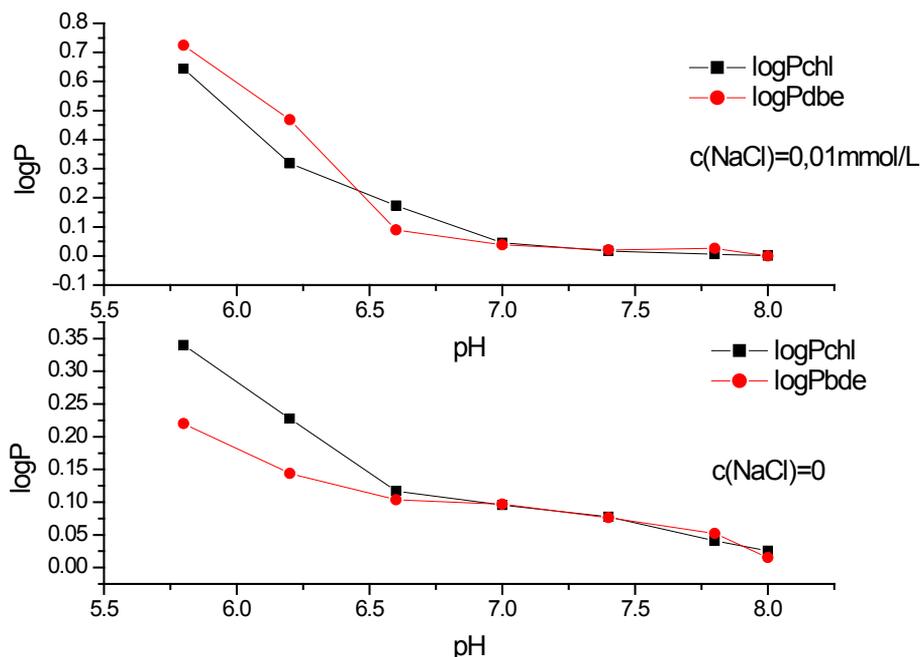


Figure 3.

It is found that, for both organic solvents, after the addition of hydroxyl group in the steroid nucleus (i.e. if the bile acid is less hydrophobic) the value of logP decreases BA(*p*) especially if more hydroxyl groups are present (Table 1). This can be explained as bile acid molecules, with more hydroxyl groups, are more stabilised in an aqueous than in organic solvent.

Table 1. LogP values between chloroform and water for NaC, NaD and NaL at different pH values

pH	logP		
	NaC	NaD	NaL
5.8	0.3402	0.9271	7.6570
6.2	0.2276	0.8810	5.0181
6.6	0.1168	0.7063	3.6124
7	0.0955	0.5329	1.6188
7.4	0.0775	0.3589	0.6672
7.8	0.0408	0.0700	0.4985
8	0.0255	0.1295	0.5974

Namely, the distribution process of bile acids can be presented in two phases. In the first phase, water molecules hydrogen bonded to the bile acids from solvation sheath are liberated, then the bile acid can move to nonpolar solvent. This can be represented with two equations:



where W stands for water molecules,  $BA_{(p)}$ ,  $BA_{(u)}$ , are bile acids present in polar phase (water) and nonpolar phases (chloroform or dibutyl ether).

Along with the increase of ionic strength, during the whole analysed range of pH values for sodium cholate, we can see the similar behaviour of the curves. The highest jump of the partition coefficient value for chloroform is when the first jump of the concentration of sodium salts (concentration of 0.05 M NaCl) occurs, while for the next jump of NaCl concentration, partition coefficient have the lower value for each examined pH values (Fig. 4). The situation is similar when logP value is measured between dibutyl ether and water but the highest jump of logP value occurs with the second jump of the concentration of sodium chloride (concentration is 0.1 M NaCl). With further increase in amount of sodium ions partition coefficient decreases (Fig. 5).

According to this we can conclude that chloroform/water system faster saturates with electrolyte ions than with dibutyl ether/water system.

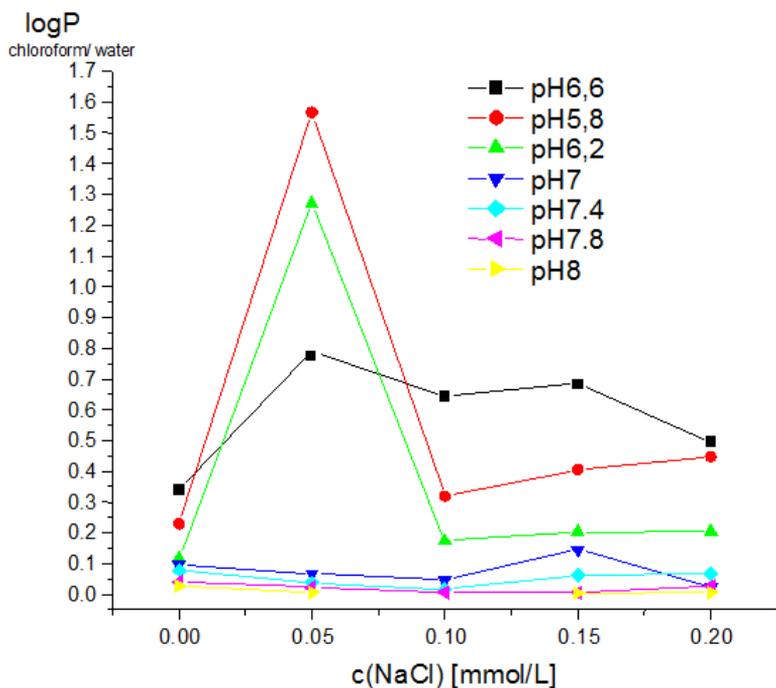


Figure 4.

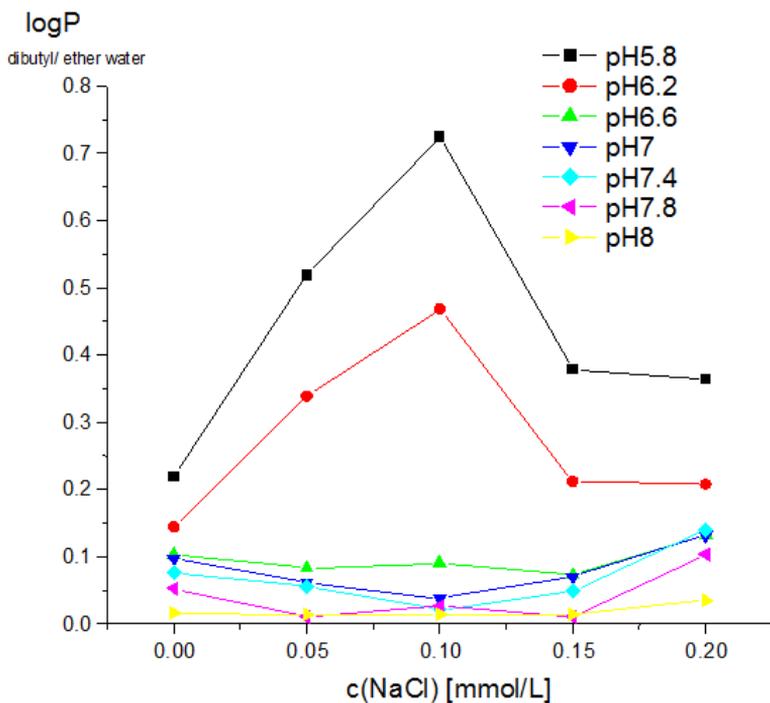


Figure 5.

## CONCLUSION

For all three analysed bile acid salts values of  $\log P$  are lower in dibutyl ether than in chloroform. At certain pH values, curves representing the dependence of partition coefficient on pH value intersect, and these are the pH values for which partition coefficients are the same for both solvents. Increasing the solution ionic strength, this intersection is shifted toward lower pH values. It is found that, for both organic solvents, after the addition of hydroxyl group in the steroid nucleus (i.e. if the bile acid is less hydrophobic) the value of  $\log P$  decreases, especially if more hydroxyl groups are present. Chloroform/water system faster saturates with electrolyte ions than with dibutyl ether/water system.

## ACKNOWLEDGMENTS

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## УТИЦАЈ СТРУКТУРЕ ЖУЧНИХ КИСЕЛИНА НА ЊИХОВ ПАРТИЦИОНИ КОЕФИЦИЈЕНТ У ДИБУТИЛ ЕТРУ И ХЛОРОФОРМУ

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**РЕЗИМЕ:** Жучне киселине добро су познати природни сурфактанти који могу да модификују пропустљивост биолошких мембрана. Логаритам партиционог коефицијента између традиционално коришћеног N-октанола и воде је мера липофилности као предиктор расподеле раствора кроз мембране. Циљ рада је да се одреде партициони коефицијенти жучних киселина између воде и хлороформа и воде и дибутил етра на различитим рН-вредностима и са додатком различитих концентрација натријумових јона, као и да се испита утицај структуре језгра жучних киселина на измерени партициони коефицијент. Партициони коефицијенти три соли жучних киселина одређивани су користећи методу мућкања док су концентрације жучних киселина одређиване након дванаест сати мешања на собној температури у воденом и органском слоју користећи реверзну фазну HPLC методу са DAD детектором на 210 nm. За све три анализиране жучне киселине вредности  $\log P$  су мање у дибутил етру него у хлороформу. На одређеним рН-вредностима криве које представљају зависност партиционог коефицијента од рН-вредности секу се и то су рН-вредности на којима су коефицијенти расподеле исти за оба растварача. Са повећањем јонске јачине раствора тачка пресека помера се ка нижим рН-вредностима. Пронађено је да за оба растварача након додатка хидроксилне групе у стероидно језгро (односно што је жучна киселина мање хидрофобна) вредности  $\log P$  опадају посебно ако је присутно више хидроксилних група. Са хлороформом као растварачем систем се брже засити јонима електролита него са дибутил етром.

**КЉУЧНЕ РЕЧИ:** дибутил етар, хлороформ, партициони коефицијент, жучне киселине