Carnitine Alkyl Ester Bromides as Novel Biosourced Ionic Liquids, Cationic Hydrotropes and Surfactants

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Abstract

Hypothesis

In contrast to anionic and nonionic amphiphilic substances, bio-based cationic ones are very rare. Cationic amphiphiles are mostly based on quaternary ammonium, pyridinium or imidazolium groups that are either badly biodegradable or have toxic residues even after degradation. In the search for green alternatives to cationic hydrotropes and amphiphiles, natural L-carnitine could be a promising candidate for a cationic headgroup.

Experiments

By esterification of carnitine in one step and with low cost, cationic molecules with alkyl chain length of n = 2 to 14 could be obtained. Their thermal properties, aggregation behaviour

and cytotoxicity were determined. Hydrophobic compounds were solubilized in their aqueous solutions and the PIT-slope method was applied to determine a relative hydrophilicity.

Findings

It was found that some pure carnitine ester bromides were liquid at room temperature and thus can be classified as ionic liquids. They are highly water-soluble, and in aqueous solutions, they showed hydrotrope or surfactant behaviour depending on their alkyl chain length. Their high hydrotropic efficiency was demonstrated by solubilising Disperse Red 13, while also biomolecules, like vanillin, could be dissolved in reasonable amounts. In all tests, they performed at least as good as the tested reference substances, while showing similar cytotoxicity towards human skin keratinocytes, thus demonstrating their potential as green functional amphiphilic molecules of positive charge.

1. Introduction

Carnitine, also called 3-hydroxy-4-*N*-trimethylaminobutyrate, is a naturally sourced quaternary ammonium compound [1]. It occurs in different biological materials, like animal tissue, plants, fruits and microorganisms, whereas mammal muscles are the richest source. In the organism, it can be partly bio-synthesized from the lysine and methionine amino acids. It is needed for fatty acid transportation through mitochondrial membranes [2,3]. As a chiral compound, *L*-carnitine is the biologically active form. It is commercially available as a nutritional supplement and supposed to enhance fat burning. Carnitine is a zwitterion in its neutral state and combines the functional groups of choline (hydroxyl group) and betaine (carboxylic group). While choline and its derivatives are well known in the field of green alternative solvents, surfactants and catalysis [4–8], carnitine has only been scarcely studied in this context up to now. This quaternary ammonium, which is authorized in food, pharmaceutics and cosmetics, is therefore a relevant candidate for the design of novel bio-based, biocompatible and non-toxic amphiphiles [9].

A lot of literature can be found on acylcarnitine compounds regarding their biological functions [10–13]. Most reports are on their formation in the organism during fatty acid transport through the inner mitochondrial membrane. Only a few studies dealing with the surfactant properties of long-chain acylcarnitine compounds have been published [14–16]. Goñi *et al.* have investigated the surfactant properties of palmitoylcarnitine, a zwitterionic amphiphile used as a biological detergent [17]. The authors report its low critical micellar concentration and its ability to solubilize biological membranes. In contrast, literature on linear carnitine amphiphiles formed by modification of the carboxyl group is rather rare. De Maria *et al.* and Cipollone *et al.* have shown the formation of cubic, hexagonal and lamellar phases as well as multilamellar vesicles by different diesters of carnitine depending on the alkyl substituents [18,19]. A detailed study of the self-assembly of carnitine dodecyl amide (*i.e.* 3-(dodecylcarbamoyl-2-hydroxypropyl)-trimethylammonium) has been published by

Patra *et al.* [20] who described the spontaneous formation of cationic vesicles in water and examined the applicability of these aggregates to form complexes with DNA for gene delivery. Another paper of this group deals with the interactions between the cationic carnitine hexadecyl amide surfactant with the protein pepsin [21].

Apart from these studies, carnitine has been rarely used for the design of amphiphilic molecules. In the present work, we have thus been interested in bromide salts of carnitine alkyl esters (**Figure 1**) with alkyl chain lengths ranging from C_2 to C_{14}.
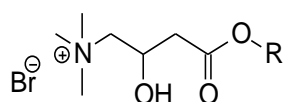
Figure 1. Molecular structure of the carnitine alkyl ester bromides; $R = C_nH_{2n+1}$ with n = 2 to 14.

Depending on the chain length, different properties and behaviours were observed, some of them typical of hydrotropes and others of surfactants. Analogous to the critical micellar concentration (CMC) that characterizes surfactants, there is a certain hydrotrope concentration beyond which the solubilization power of hydrophobic compounds significantly increases. This concentration is often called the minimum hydrotrope concentration (MHC) [22].

Beside this, some ionic hydrotropes can also exhibit ionic liquid properties as reported by several authors. For instance, Claudio *et al.* reported the hydrotropic activity of several ionic liquids based on imidazolium, pyridinium, piperidinium, quaternary ammonium and phosphonium compounds as well as their ability to solubilize some antioxidant biomolecules. In this way, they established a connection between the concept of ionic liquids and the hydrotropic solubility phenomenon [23]. However, it should be noted that none of these positively charged headgroups are biobased.

In the present study, a series of alkyl carnitine ester bromides from C_2 to C_{14} has been prepared in a simple one-step synthesis. Since esters are relatively easy to cleave, the (bio-)

degradation products of them will mainly consist of products found in nature and as thus should be more easily biodegradable.

The pure compounds were characterized for their thermal properties. Their aqueous phase behaviour has been investigated by surface tension measurements and dynamic light scattering. The ability of the short-chain derivatives to solubilize the Disperse Red 13 hydrophobic dye was studied in order to examine their hydrotropic action and the solubility of vanillin was determined to demonstrate their usefulness in application. For such a purpose, the cytotoxicity of the newly developed compounds has also been determined and compared to two conventionally used cationic surfactants (DTAB, CTAB), one typical hydrotrope (SXS) and one ionic liquid ([C_1C_4Im]Br). Finally, the carnitine ester compounds were classified according to their hydrophobic/lipophilic behaviour using the PIT-slope method [24] which is more powerful and more robust than the well-known HLB of Griffin [25]. The results are discussed in terms of properties related to chain length and potential applications of these novel carnitine-based compounds.

2. Experimental Section

2.1. Materials

L-carnitine (99%), bromoethane (98%), 1-bromooctane (98%), 1-bromodecane (98%) and 1-bromododecane (98%) were purchased from Alfa Aesar. 1-Bromobutane (99%), 1-bromohexane (98%), 1-bromotetradecane (97%), dodecyltrimethylammonium bromide (99%) and 1-butyl-3-methylimidazolium bromide (> 97%) were delivered by Sigma Aldrich. Disperse Red 13 came from Acros and vanillin (> 99%) from Roth. Sodium xylene sulfonate (> 90%) and cetyltrimethylammonium bromide (> 99%) were purchased from Fluka. NMR solvents D_2O, d-DMSO and $CDCl_3$ had an isotopic purity of 99.95% and were from Eurisotop.

2.2. Synthesis of carnitine alkyl ester bromides [C_nCar]Br (n = 2 to 14)

The carnitine ester bromides, abbreviated as **[C$_n$Car]Br,** were synthesized *via* the reaction of L-carnitine with 1-bromoalkanes (R = C$_n$H$_{2n+}$1 with n = 2 to 14) in acetonitrile under reflux. L-Carnitine (1 equiv.) and 1-bromoalkane (1.5 equiv.) were mixed in acetonitrile under inert conditions (argon-gas) before heating under reflux for at least 12 h. The reaction was monitored by NMR analysis. After evaporation of the solvent, the crude product was washed several times with diethyl ether and dried under vacuum. The product yields were > 90%.
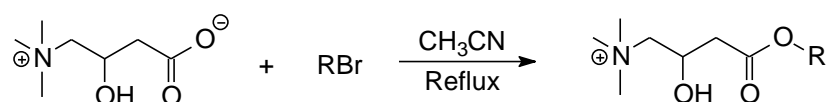
Figure 2. One-step synthesis of carnitine alkyl ester bromides; R = C$_n$H$_{2n+1}$ with n = 2, 4, 6, 8, 10, 12, 14.

All NMR data are given in the Supporting Information (**S2**).

2.3. Surface tension measurements

Surface tension measurements were performed with a KRÜSS K100CC tensiometer with Wilhelmy plate geometry at room temperature. Before each measurement, the platinum plate was intensively cleaned and dried to avoid any contamination. The sample solutions were prepared in volumes between 10 and 50 mL in millipore water and surface tension was measured after equilibration. The resulting values were mean quantities of at least two measurements. The critical aggregation (micelle) concentrations (CAC and CMC) and the minimum surface tensions (σ_{min}) were taken from the surface tension curves.

2.4. Dynamic light scattering (DLS)

The concentrations of the DLS samples were chosen to be approximately 0.1 M (see Table 2) above the CMC of each compound. Exceptions were made for the reference substances, where either no CAC could be clearly determined or where there was restricted water solubility. The solutions were prepared in test tubes and approximately 3.5 mL solution were filtered with a 0.2 µm PTFE membrane filter in order to remove dust particles. The samples

were poured into cylindrical light-scattering cells with 10 mm outer diameter. They were then placed in a temperature controlled toluene bath of 25 °C, which was part of the CGS-3 goniometer system from ALV (Langen, Germany) equipped with an ALV-7004/FAST Multiple Tau digital correlator and a vertical-polarised 22-mW HeNe laser (wavelength λ = 632.8 nm). The homodyne correlation functions $\langle I(0)I(\tau)\rangle$ (or $G^1(\tau)$, τ being the correlator delay time) were recorded for 300 s at a 90° angle [26]. Kinematic viscosities and refractive indices of the sample solutions were determined. The dynamic viscosities η were measured with a rolling ball viscosimeter AMVn from Anton Paar (Graz, Austria) at 25 °C (298.15 K). The refractive indices n were obtained by an Abbe Refractometer AR3-AR4 from A. Krüss Optronic GmbH (Hamburg, Germany) at 25 °C (298.15 K) with a wavelength of 589 nm. Density measurements were performed on a DMA 5000M densitometer from Anton Paar (Graz, Austria).

2.5. Solubilization of Disperse Red 13

The solubilization of the hydrophobic dye Disperse Red 13 was measured using an Agilent Technologies Varian Cary 60 UV-visible spectrophotometer. To this purpose, aqueous stock solutions (ca 2 M) of **[C$_n$Car]Br** were prepared with millipore water and Disperse Red 13 was added until saturation was reached and powder remained in suspension. The solutions were stirred for 24h at 25 °C. To eliminate excess hydrophobic dye, each solution was filtered and the amount of dissolved Disperse Red 13 was determined by UV-visible absorption at 503 nm, where the absorbance was in the range between 0.5 and 2.

2.6. Solubilization of vanillin

[C$_n$Car]Br aqueous solutions were prepared at different concentrations taking into account their MHC values and the water-solubility of the compound. Vanillin was added in excess to each solution. The solutions were stirred at 700 rpm at 25 °C for at least 72 h until saturation. The samples were centrifuged in an EBA III centrifuge of Hettich (Tuttlingen, Germany) for

25 min with maximum speed to separate the solid excess of vanillin. The liquid phase was collected and diluted in ultrapure water. The solubilized amount of vanillin was quantified by UV-visible spectroscopy using a Lambda 18 UV/Vis spectrometer from Perkin Elmer (Neuried, Germany) at 280 nm. A calibration curve was previously established with ethanol as solvent (Supporting Information, **Fig. S3**). Reference samples with L-carnitine, SXS and **[C$_4$C$_1$Im]Br** were prepared and measured in the same way.

2.7 Cytotoxicity tests

Cytotoxicity tests were performed in vitro with human skin keratinocytes (HaCaT cells) in form of a PrestoBlue assay. Detailed information can be found in the Supporting Information (**S1.6**).

2.8. PIT-slope method

The reference system used for this method was the tetraethyleneglycol monododecyl ether C$_{10}$E$_4$/*n*-octane/water system. For all samples the water fraction f_{water} was constant at 0.5. The detailed procedure is given in the Supporting Information (**S1.7**).

3. Results and Discussion

3.1. Temperature-dependent phase behaviour of the pure [C$_n$Car]Br compounds (n = 2 to 14)

Ionic liquids (ILs) are salts with low melting temperature. Of particular interest are room temperature ionic liquids (RTILs). They often show high solubility and/or miscibility, because the crystallization energy does not need to be payed when they are already in a liquid state.

. They are characterized by a wide liquid range and a negligible vapour pressure [27,28]. Thermal properties (*i.e.* thermal phase transitions and thermal stabilities) of the **[C$_n$Car]Br** compounds were determined and evaluated regarding ionic liquid characteristics. Thus, melting (T_m) and glass transition (T_g) temperatures were studied by differential scanning calorimetry (DSC) and degradation (T_{deg}) temperatures by thermogravimetric analysis (TGA)

(**Table 1**).

Table 1. Degradation temperature T_{deg}, melting temperature T_m and glass transition temperature T_g of **[C$_n$Car]Br** compounds for different chain lengths.

n	T_{deg} [°C]	T_m [°C]	T_g [°C]
2	190	129	/
4	189	/	-22
6	187	/	-29
8	185	34	/
10	235	59	/
12	239	69	/
14	241	71	/

The results in **Table 1** show that **[C$_2$Car]Br** with a melting temperature of 129 °C cannot be considered as an IL, while **[C$_4$Car]Br** and **[C$_6$Car]Br** are room temperature ILs (RTILs) with glass transitions at -22 and -29 °C respectively. Glass transitions typically occur for ILs, as they organize into amorphous states upon cooling. The formation of a crystal lattice is hindered due to steric reasons [29]. In contrast, the longer chain carnitine bromides with n = 8 to 14 have melting points of 34, 59, 69 and 71 °C, respectively. Per definition, they are ILs, too. Their different temperature-dependent phase behaviours can be explained by an increase of van der Waals interactions with increasing alkyl chain length [30]. There is an interesting break in degradation temperatures, where **[C$_n$Car]Br** compounds with n ≥ 10 seem to be significantly more stable towards temperature than those with n ≤ 8. This can be attributed to stronger van der Waals interactions present in the long chain derivatives.

3.2. Self-aggregation of [C$_n$Car]Br compounds (n = 2 to 14) in water

With an amphiphilic and ionic structure, a certain organization of the alkyl carnitine esters in water can be expected, similar to that of hydrotropes in the case of short alkyl chains and of

surfactants in the case of longer alkyl chains. As a consequence, either pure or in aqueous solution, such molecules are indeed expected to exhibit some solubilizing properties of hydrophobic compounds: solvent-like if we consider the pure IL and hydrotropic or micellar in aqueous solution. Hence, the self-aggregation behaviour of the series of carnitine ester bromides **[CₙCar]Br** was first studied by measuring the equilibrium surface tensions (σ) in dilute aqueous solutions at different concentrations (**Figure 3**).
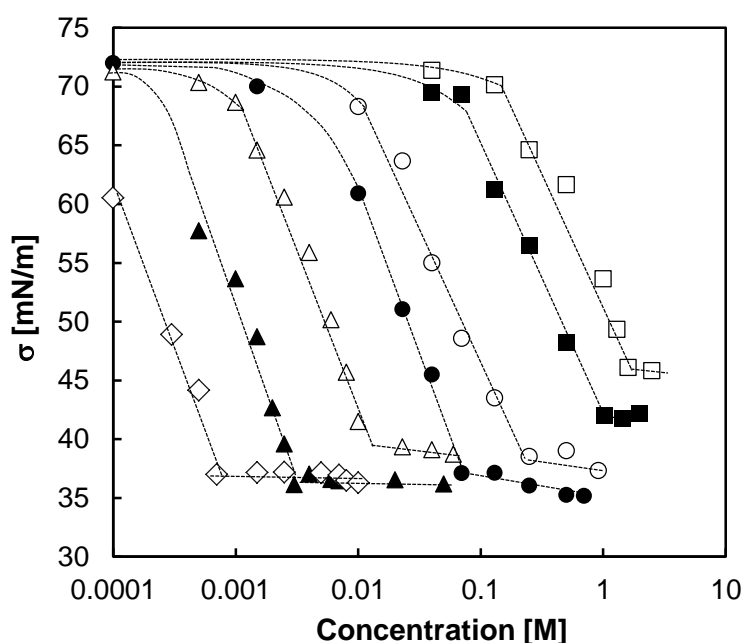
Figure 3. Surface tension curves of **[CₙCar]Br** compounds with n = 2 (□), 4 (■), 6 (○),8 (●), 10 (△),12 (▲) and 14 (◇).

As shown in **Figure 3**, the surface tension curves exhibit the typical characteristics of amphiphilic molecules with a more or less clear break in the surface tension reduction. With increasing concentration, the surface tension decreases until a certain point, the critical aggregation concentration (CAC) or the critical micellar concentration (CMC) for true surfactants, which are specific for every amphiphilic species at a given temperature. Aggregation state, water-solubility, CAC and minimum surface tension σ_{CAC} of all compounds are given in **Table 2**. The surface excess concentration (Γ_m) is calculated only for the surfactants which CMC are below 0.01M (See Supplementary Information)

Table 2. Aggregation state, water-solubility, critical aggregation concentration (CAC), minimum surface tension σ_{CAC} and surface excess concentration of **[C$_n$Car]Br** compounds.

[C$_n$Car]Br	2	4	6	8	10	12	14
State at rt	solid	liquid	liquid	liquid	solid	solid	solid
water-solubility [M]	> 10	> 10	5.9	5.3	2.4	1.3	0.65
CAC [M]	1.8	1.0	2.1×10^{-1}	9.0×10^{-2}	1.4×10^{-2}	2.7×10^{-3}	7.1×10^{-4}
σ_{CAC} [mN/m]	45.5	41.9	37.3	35.2	38.7	36.2	36.2
$\Gamma_m \times 10^{10}$ [mol/cm^2]	-	-	-	-	2.8	3.3	4.3

The CAC values for the **[C$_n$Car]Br** compounds with n = 2 to 14 range from 1.8 to 7.1 x 10^{-4} M, respectively. Th+us, with increasing alkyl chain length and therefore increasing hydrophobicity, the CAC decreases and the surfactant efficiency, that goes along with the surfactant concentration necessary for reaching a minimum surface tension, increases [31]. The CACs of the long chain carnitine bromides are in the millimolar region that is typical of the CMC of a charged surfactant. This critical threshold indicates the start of the formation of micellar aggregates in the water bulk phase [32]. With CMC values of 1.4×10^{-2}, 2.7×10^{-3} and 7.1×10^{-4} M, respectively, **[C$_{10}$Car]Br,** **[C$_{12}$Car]Br** and **[C$_{14}$Car]Br** show surfactant efficiencies in the range of the common cationic surfactant cetyltrimethylammonium bromide (CTAB) with a CMC of $\approx 1 \times 10^{-3}$ M [33], thus presenting a relevant green alternative to such petro-based cationic surfactants still used as conditioning agents for example. In contrast, the short chain carnitine bromides have their CACs in the molar range, which is typical of the minimum hydrotropic concentration (MHC) of a hydrotrope. They are unlikely to form well-defined spherical micelles, as their hydrophobic tail is too short [34].

As expected, there is a linear relationship between log (CAC) and the alkyl chain length n (see Supporting Information, **Fig. S2**). This is in accordance with the observations of Klevens who first reported this linear relationship for homologous series of surfactants [35]. In contrast,

Eastoe *et al.* found a distinct kink in an analogous plot for *n*-alkylbenzoates between n = 0 to 3 and n = 4 to 8. This phenomenon was related to a definite break between hydrotropic and surfactant behaviours of these species [36]. However, according to the CAC values obtained for the **[C$_n$Car]Br** compounds, a continuous transition from hydrotrope- to surfactant-like behaviour is observed with increasing chain length.

The effectiveness of a surfactant is independent of the surfactant concentration and only depends on the minimum surface tension that can be reached: the lower the surface tension, the higher the effectiveness [35]. The minimum surface tension shows a chain length dependence according to alkyl chain densities: the longer the alkyl chain, the closer the surfactant can pack on the surface, the lower is the surface energy [35,37]. The increase of the alkyl chain length values slightly increases the surface excess concentration (Γ_m), indicating a closing packing in the air/water interface. In this study, the surface tension values at the CAC were taken as minimum surface tension σ_{CAC}. While significantly higher σ_{CAC} values were determined for n = 2, 4, they were approximately at the same level for n = 6-14. Thus, although higher concentrations of middle-chain hydrotropes/surfactants (*e.g.* n = 6) are necessary for the reduction of the surface tension of water, the minimum surface tension can be as low as for long-chain compounds (*e.g.* n = 14), *i.e.* while the efficiency shows strong chain length dependence, the effectiveness can be similar [38–40]. The maximum surface excess concentrations of carnitine alkyl ester bromides are higher than the reported data for 1-alkyl-3-methylimidazolium [REF: 2007_LANG_Dong] with the same alkyl chain (n=10 and n=12). Carnitine polar head of a single surfactant molecule had a smaller area at the air-water interface.

To summarize, an alkyl chain length dependence of CAC and σ_{CAC} values of **[C$_n$Car]Br** compounds can be observed. CACs of short chain carnitine bromides are in a range typical of hydrotropes, while those of the long chain compounds are in a range typical of surfactants. However, there is no clear distinction between hydrotropes and surfactants, rather a

continuous transition when the chain length is increased. Compared to other cationic surfactants as alkyl-3-methylimidazolium , alkylpyridinium bromides or alkyltrimethylammonium bromides with alkyl chain between 10 and 14 [REF: 2007_LANG_Dong] the CMC and the minimum surface tension of alkyl carnitines are lower.

3.3. Characterization by dynamic light scattering of aggregates formed by the [CₙCar]Br compounds (n = 2 to 14)

Dynamic light scattering (DLS) measurements were carried out to further characterize the **[CₙCar]Br**/water binary systems. The concentrations of the DLS solutions were chosen at CMC + 0.1 M. **Figure 4** gives the self-correlation functions for the different compounds from n = 2 to 14. The reference solutions of **SXS** and **[C₄C₁Im]Br** had concentrations of 0.5 M and 3.5 M, respectively. **DTAB** and **CTAB** solutions were prepared with 0.111 and 0.015 M, respectively.
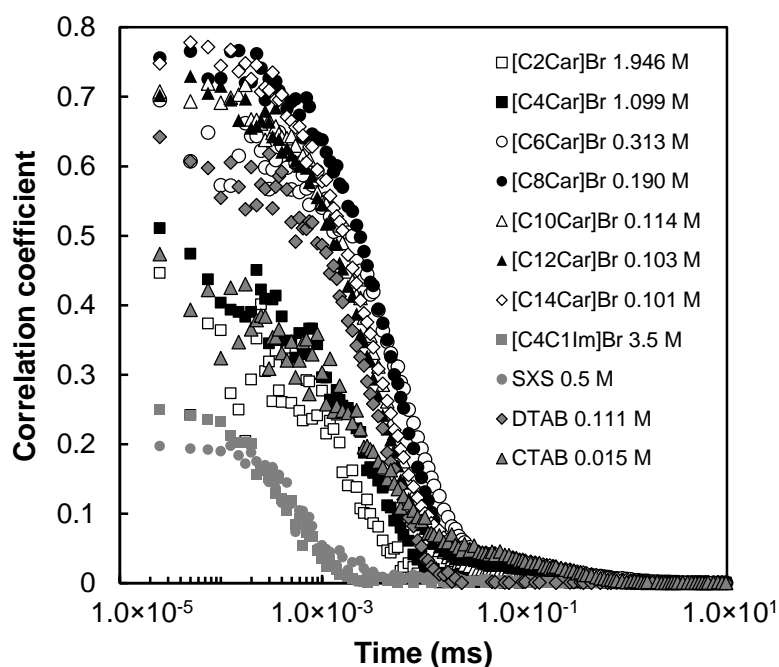
**Figure 4.** Time-dependent self-correlation functions as obtained by DLS for the **[CₙCar]Br/**water binary system at 25 °C at concentrations equal to CMC + 0.1 M.

As can be seen in **Figure 4**, well-defined correlation functions are obtained for n = 6 to 14. The correlation functions became less pronounced with decreasing alkyl chain length. This finding suggests that the long-chain compounds are more likely to form aggregates than the short-chain ones, which, nevertheless, show also a moderate tendency to form aggregates, as we can expect for hydrotropes [22]. As for the other measured properties of this series of surface-active molecules, there is no significant break in the evolution when the chain length is increased. **SXS** and **[C₄C₁Im]Br** aqueous systems were measured as reference systems. According to the results obtained from DLS, they show a weak aggregation behaviour similar to **[C₂Car]Br**. While the autocorrelation function of **DTAB** is similar to the long-chain carnitine derivatives, that of **CTAB** is in the range of **[C₆Car]Br.** Usually, a distinct self-assembly could be expected for **CTAB** with its C_{16} carbon chain. However, this result can be attributed to the low concentration of the **CTAB** solution (0.015 M) due to its low water solubility. *Please note that as usual, due to the significant ionic strength of the samples, the calculation of micelle hydrodynamic radii from the autocorrelation function via Stokes-Einstein-equation did not yield reasonable results [41].*

3.4. Hydrotropic efficiency of short-chain [CₙCar]Br compounds (n = 2 to 8)

The hydrotropic efficiency of the carnitine ester bromides **[CₙCar]Br** (n ≤ 8), *i.e.* their ability to enhance the solubility of a hydrophobic, sparingly water-soluble compound [42], was evaluated by measuring the solubilization of the hydrophobic dye Disperse Red 13 (*i.e.* 2-[4-(2-chloro-4-nitrophenylazo)N-ethylphenylamino]ethanol), which can be readily followed by UV/visible spectroscopy. In **Figure 5**, the absorbance of the dye at 503 nm has been plotted against the hydrotrope concentrations of the **[CₙCar]Br** with n = 2 to 8 as well as **SXS**, **[C₄C₁Im]Br** and L-carnitine. The measured absorbance is proportional to the amount of Disperse Red 13 dissolved.
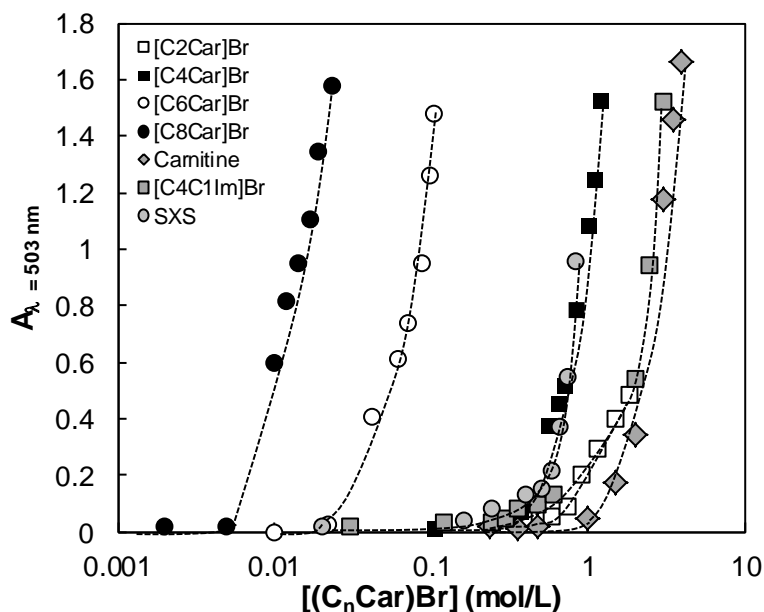
Figure 5. Hydrotropic solubilization of Disperse Red 13 in **[CₙCar]Br** (n = 2, 4, 6 and 8) aqueous solutions.

It can be observed that the increase in solubilization starts after a certain threshold concentration. From the Disperse Red 13 solubilization curves, these concentrations cannot be determined precisely but they can be roughly estimated. They are correlated to the hydrotrope efficiency for solubilizing hydrophobic substances. The following order can be observed: **[C₈Car]Br > [C₆Car]Br > [C₄Car]Br > [C₂Car]Br**. This means that hydrotrope efficiency increases with chain length [43].

Contrary to what could be expected, the critical values in these ternary systems seem to be smaller than for the binary systems (**see Figure 3**). We assume that the hydrophobic component promotes aggregation and the molecules start to self-assemble earlier in the presence of a third hydrophobic component.

Nevertheless, the mechanism of hydrotropic solubilization is not exactly clarified yet and different theories are reported. One is based on the existence of water-hydrotrope interactions that might drive hydrotropic solubilization [44]. Another one describes the formation of low-stochiometric solute-hydrotrope complexes as the origin of hydrotropy [45]. However, we believe that for carnitine ester bromides, the mechanism resembles a micellar solubilization

process, as we observed self-assembly of carnitine esters in aqueous solution for all chain lengths in DLS measurements (see section 3.3). Nevertheless, the addition of a third hydrophobic compound might enhance aggregation and also hydration might probably be an issue. As hydrotropic solubilization is driven by the complex interplay of interactions between water, solute and hydrotrope, further investigations on these systems have to be done to clarify the system structure.

Likewise, Disperse Red 13 solubilization tests were performed with **SXS**, **[C$_4$C$_1$Im]Br** and L-carnitine. **[C$_4$Car]Br** seems to have a solubilization efficiency (threshold concentration) similar to the classical hydrotrope **SXS**. However, the carnitine derivative shows better performance, as the amount of solubilized Disperse Red 13 is higher. All **[C$_n$Car]Br** solutions were more efficient for Disperse Red 13 solubilization than **[C$_4$C$_1$Im]Br** and L-carnitine. It was interesting that in the surface tension curves of **[C$_4$C$_1$Im]Br** and L-carnitine (Supporting Information, **Fig. S1**), no CAC could be determined, whereas from the solubility curve the formation of aggregates can be assumed. This would mean that these compounds form aggregates only in the presence of a hydrophobic compound.

3.5. Solubilization of vanillin

Vanillin, or 3-methoxy-4-hydroxy-benzaldehyde, is a biomolecule occurring in green vanilla beans in high amounts. This molecule belongs to one of the most popular flavouring materials and is used in food, beverage, pharmaceutical and cosmetic industry. In addition to that, it exhibits antioxidant, antimicrobial and radical-scavenging properties and is therefore highly interesting as a preservative in all kinds of chemical formulations. Its water-solubility is quite low ($\approx$ 0.06 M) and extraction processes typically use volatile organic solvents [46,47]. Thus, we have investigated the solubility of vanillin in water in the presence of increasing amounts of the **[C$_n$Car]Br** compounds in comparison with **L-carnitine**, **SXS**, **[C$_4$C$_1$Im]Br, DTAB** and **CTAB** solutions as references. Results are depicted on **Figure 6**.
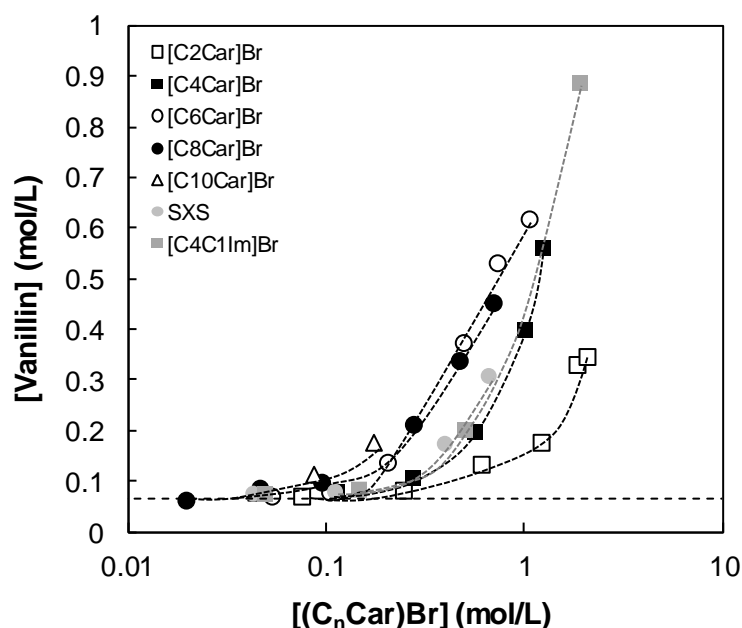
Figure 6. Solubility of vanillin at room temperature in aqueous solutions of **[C$_n$Car]Br**. For sake of clarity, only the curves for n = 2 to 10 are shown in comparison with **SXS** and **[C$_4$C$_1$Im]Br.** Curves for n = 12, 14, DTAB, CTAB and L-carnitine can be seen in **Supporting Information (Fig. S4)**. Dotted line indicates water-solubility of vanillin in water.

While unesterified L-carnitine shows the smallest vanillin solubilization capacity of all tested substances (0.12 M at 2.7 M), **[C$_n$Car]Br** ester compounds clearly enhance the water-solubility of vanillin. Though the long-chain **[C$_n$Car]Br** compounds are restricted by their lower water-solubility (see Table 2), aqueous solutions of the short-chain derivatives show excellent vanillin solubility. Indeed, aqueous solutions of **[C$_4$Car]Br** and **[C$_6$Car]Br** (1 mol/L) solubilize ≈ 0.40 and 0.62 M of vanillin respectively and therefore increases its solubility about tenfold compared to pure water.

High solubility efficiency could also be reached with the reference hydrotrope **SXS** (0.3 mol/L vanillin with 0.65 M SXS) and with the **[C$_4$C$_1$Im]Br** ionic liquid (0.88 M vanillin with 2 M [C$_4$C$_1$Im]Br). For these hydrotropes, the solubilization is probably favoured by π interactions of the aromatic ring of vanillin with the imidazolium and aromatic rings of [C$_4$C$_1$Im]Br and SXS respectively. Compared to **[C$_4$C$_1$Im]Br**, **SXS** is however restricted by

its lower water-solubility. Especially the conventionally used surfactant **CTAB** was restricted through its own water-solubility, so that the increase of vanillin water-solubility by **CTAB** was only small. **DTAB**, in contrast, performed similarly to the longer chain carnitine derivatives (n = 12 and 14), *i.e.* $\approx$ 0.2 M vanillin for 0.2 M of surfactant.

3.6. Cytotoxicity

The cytotoxicity of all **[C_nCar]Br** compounds was evaluated *in vitro* with human skin keratinocytes (HaCaT cells) and compared to that of **SXS**, **[C_4C_1Im]Br, DTAB** and **CTAB**. The resulting EC_{50} values correspond to the concentrations at which 50% of the examined cells survived. Results are reported on **Figure 7**. The corresponding cell viability data can be found in the Supporting Information (**Fig. S5**).
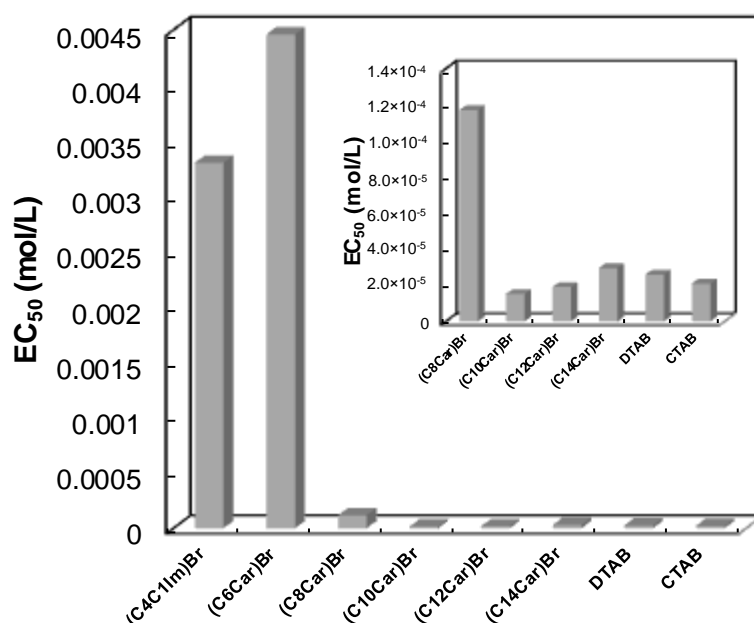
Figure 7. EC_{50} values in [M] for **[C_nCar]Br** compounds and two common cationic surfactants (literature values for DTAB and CTAB are from Yu *et al.* [48]).

While **[C_2Car]Br**, **[C_4Car]Br** and **SXS** did not show any cytotoxicity in the measured concentration range, the EC_{50} values of **[C_{10}Car]Br**, **[C_{12}Car]Br** and **[C_{14}Car]Br** with 15, 19 and 30 µM were in the same range as the well-known cationic surfactants **DTAB** and **CTAB**

with 26 and 21 µM [48]. This is an expected result because the toxicity is mainly given by the hydrocarbon-chain length, as it is well known for many other compounds.

With EC_{50} values of 0.1 and 4.5 mM, respectively, the middle-chain compounds **[C$_8$Car]Br** and **[C$_6$Car]Br** are less toxic than the long-chain ones. Also, the reference substance **[C$_4$C$_1$Im]Br** exhibits low cytotoxicity compared to the long chain carnitine bromides. However, in comparison to its carbon-chain analogue **[C$_4$Car]Br,** the carnitine compound shows better cytotoxicity characteristics.

In fact there are several studies on the cytotoxicity of quaternary ammonium compounds and cationic surfactants [49,50]. It seems clarified that surfactants tend to interact with phospholipid cell membranes where they affect their physical properties and function. At high surfactant concentration and long exposure, it comes to an association of components of the cell membrane and the surfactants and therefore to cell lysis. However, for cationic surfactants, cytotoxicity was also observed at concentrations that were too low to cause cell lysis. Inácio *et al.* reported that the reason for this cytotoxicity was a mitochondrial dysfunction induced by quaternary ammonium compounds [50]. Therefore it is questionable if the cytotoxicity of cationic surfactants, that are quaternary ammonium species in most cases, can be fully avoided. Nevertheless, it can be reduced by decreasing the alkyl chain length. Developing carnitine cationic surfactants that have high surfactant efficiency even with lower alkyl chain length might be an approach towards less toxic cationic surfactants.

3.7. Hydrophilic/lipophilic balance of the [C$_n$Car]Br compounds (n = 6 to 14) according to the PIT-slope method

Finally, the hydrophilic/lipophilic balance of the carnitine alkyl ester bromides **[C$_n$Car]Br** was determined using the PIT-slope method [24]. More robust than the well-known HLB of Griffin, this method is based on monitoring the evolution of the phase inversion temperature (PIT) of the well-defined $C_{10}E_4$/*n*-octane/water reference system upon addition of a second amphiphile, S$_2$, what results in an increase or decrease of the emulsion PIT. For nonionic

surfactants, it has been shown that a linear regression allows a correlation between the PIT and the molar fraction x_2 of S_2 providing the so-called PIT-slope as a descriptor of the hydrophilic/lipophilic balance of S_2. According to Shinoda *et al.*, the PIT corresponds to the temperature where the hydrophilic/lipophilic character of a nonionic ethoxylated surfactant flips over [51]. The dehydration of the polyoxyethylene units during heating leads to the inversion from an oil-in-water (o/w) to a water-in-oil (w/o) emulsion. This process can be monitored by measuring the emulsion conductivity [52,53]. The $C_{10}E_4$/*n*-octane/water is used as a reference system as its initial PIT is close to room temperature [54]. By addition of S_2, the PIT changes positively or negatively relative to the reference system as follows: positive values for the PIT deviation indicate a more hydrophilic character and negative values a less hydrophilic character of S_2 compared to $C_{10}E_4$ [24]. Thus, although this method does not provide absolute values, it delivers a comparative criterion that illustrates the activity of a molecule at the water/oil interface and its relative hydrophilicity. **Figure 8** shows the evolution of the PIT at different mole fractions of the **$[C_nCar]Br$** compounds from n = 6 to 14 in comparison with already reported alkyltrimethylammonium bromides **$[C_n]Br$** [55].
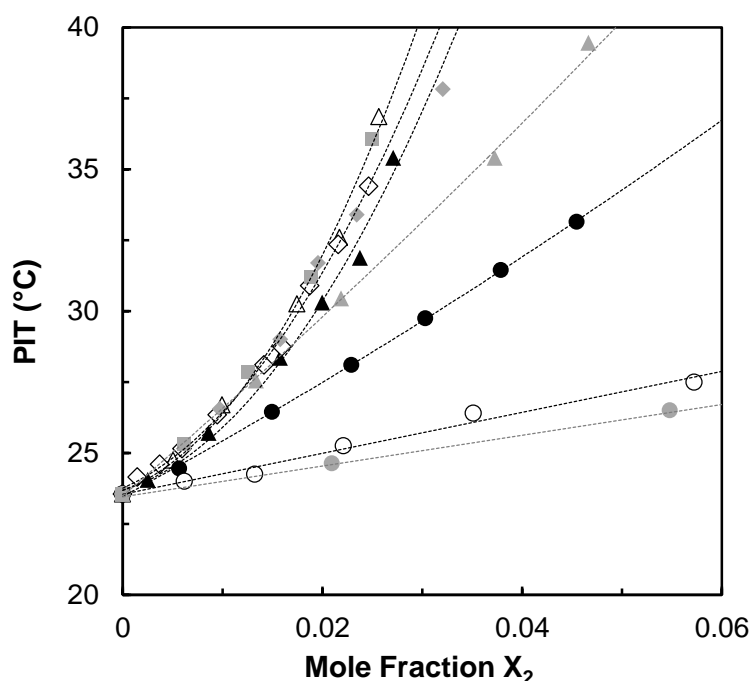
Figure 8. Phase inversion temperature of the system 3 wt.% $C_{10}E_4$/octane/10^{-2} M NaCl$_{aq}$ at f_w

= 0.5 *versus* molar fraction x_2 of **[C$_n$Car]Br** with n = 6 (○), 8 (●), 10 (△), 12 (▲) and 14 (◇) in comparison with alkyltrimethylammonium bromides **[C$_n$]Br** with n = 8 (⬤), 10 (▲), 12 (■) and 14 (◆) from ref [55].

As can be seen on **Figure 8**, all surfactants have a positive impact on the PIT meaning that, they are more hydrophilic than $C_{10}E_4$. It is noteworthy that the addition of S_2 was restricted, as with higher amounts, the emulsion did not invert within the experimentally used temperature range of up to 60 °C. As far as carnitine derivatives are concerned, the linear fits match the obtained data for **[C$_6$Car]Br** and **[C$_8$Car]Br** very well. In contrast, longer-chained carnitine compounds with n = 10, 12, 14 do not exhibit a linear behaviour. Indeed, as already reported, the linearity for nonionic surfactants S_2 ($R^2 > 0.99$) is better than for ionic surfactants ($R^2 > 0.95$) [55]. This can be attributed to the presence of a non-ideal surfactant mixture of ionic and nonionic surfactants due to strong interaction at the interface [56–59]. However, linearity can be considered at low x_2 values and, by taking into account only the data for which the PIT is lower than 30 °C, we obtain for the carnitine compounds the following order: **[C$_6$Car]Br (72) < [C$_8$Car]Br (207) < [C$_{12}$Car]Br (310) < [C$_{10}$Car]Br (316) < [C$_{14}$Car]Br (317)**. This ranking clearly indicates a typical surfactant behaviour for **[C$_n$Car]Br** with n = 10, 12 and 14 which behave very closely: they are mainly localized in the interfacial layer impacting the PIT according to their hydrophilicity. It is worth noting that when the molar fraction x_2 increases, the deviation from linearity is more pronounced for **[C$_{10}$Car]Br** compared to **[C$_{12}$Car]Br** and **[C$_{14}$Car]Br**. This probably reflects a stronger interaction with $C_{10}E_4$ at the interface as already shown in our previous work with $C_{10}E_j$ and [DiC$_{10}$]Cl [59]. In contrast, the short-chain ester compounds with n = 6, 8 do not show proper surfactant properties, as they are partitioned between the aqueous phase and the interface.

Compared to typical cationic surfactants reported in the literature [55], it is interesting to note that **[C$_6$Car]Br** behaves almost as **[C$_8$]Br** and that **[C$_{10}$Car]Br** behaves almost as **[C$_{12}$]Br**. In

other words, they would have a similar hydrophilicity which tends to show that the carnitine headgroup, though containing ester and hydroxyl groups in its structure, does not bring as much hydrophilicity as expected, probably due to intramolecular hydrogen bonding between the two functions. Hence, with four additional carbon atoms, the carnitine moiety would be equivalent to two CH_2 groups. This could be of interest in the use of C_6 or C_{10} alkyl chains instead of C_8 or C_{12} ones respectively for designing surfactants with same functional properties.

4. Conclusion

In this study, L-carnitine has been considered as natural cationic building block in the search for novel green amphiphilic compounds. The conversion of L-carnitine into functional molecules (carnitine alkyl ester bromides) was done in a simple, inexpensive one-step synthesis. Some of the resulting esters turned out to be room temperature ionic liquids. However, this work focused on self-aggregation and solubility behaviour of the corresponding aqueous solutions as well as the hydrophilicity of the resulting amphiphiles.

In a homologous series with alkyl chain length n = 2-14, a progressive transition from hydrotropic to surfactant behaviour was observed via DLS and surface tension measurements, different to what has been found for sodium alkylbenzoates [36]. However, according to the PIT slope method used to determine the surfactant hydrophilicity, only carnitine esters with alkyl chain length > 8 showed real surfactant properties, as they were thought to be mainly located in the interfacial layer.

All results were compared to the conventionally known hydrotrope SXS, to [C_4C_1Im]Br - a typical ionic liquid with hydrotropic properties, and to two well-established cationic surfactants, DTAB and CTAB. The comparison shows that the newly developed compounds can keep up with conventional solubilizers in terms of solubility performance and cytotoxicity, where short chain carntitine esters did not show any cytotoxicity at all.

That the cytotoxicity of cationic surfactants depends mainly on the length and the nature of the alkyl chain and to a much lesser extent on the headgroup, has been known before [60]. In this respect, no advantage can be respected. However, in contrast to other cationic surfactants, the synthesis is very simple and the carnitine molecule is available industrially through fermentation in biotechnological processes [61]. Further, during biodegradation, the cleavage of the ester bond should deliver natural products that are much less toxic than those obtained when classical cationic surfactants are degraded. However, this will be the subject of further research and remains speculation for the moment.

Concerning potential application in colloidal formulations, like cosmetics, food or pharmaceutics, the carnitine ester bromides represent a wide spectrum of properties that can be adjusted to the required properties by changing the alkyl chain length. Especially short chain compounds that did not exhibit cytotoxicity in the measured concentration range, might have a certain potential, especially in mixtures with anionic surfactants. But before, and further to deeper toxicity and biodegradability studies, full phase diagrams must be measured and the underlying structures must be elucidated.

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