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Partition of antioxidants available in biowaste using a green aqueous biphasic system

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ABSTRACT

The most common feedstocks for natural antioxidants, *i.e.*, chemicals which delay the oxidative damage by reactive oxidative species, are fruits and vegetables, but using food as raw material raises moral issues and contributes to several environmental and social problems, such as larger farming areas and global hunger. Therefore, the use of antioxidant-rich biowaste is a more feasible option for the production of dietary supplements, contributing simultaneously to a more circular economy and to more sustainable therapeutics. In this work, the partition of epicatechin and of vitamins B9, B12 and C was conducted, for the first time, in the green Aqueous Biphasic System (ABS) {ethyl lactate (1) + trisodium citrate (2) + water (3)} at 298.15 K and atmospheric pressure for future valorisation of common biowastes, such as fruit pomaces and vegetable peals. The largest partition coefficients (K) and extraction efficiencies (E) were obtained for vitamin B12 ($K = 55\ 44$, $E = 98\ 5$ %) and epicatechin ($K = 5\ 44$, $E = 84\ 8$ %) for the longest tie-line (TLL = 69 68 %), while, in the same conditions, vitamin B9 ($K = 1\ 48$, $E = 61\ 6$ %) and vitamin C ($K = 0\ 44$, $E = 32\ 2$ %) presented smaller affinity for the ethyl lactate-rich phase. These results were obtained with low biomolecule mass losses in quantification and after a careful study of the biomolecule stages present for each pH value.

1. Introduction

Even though malnutrition is a global health issue, underdeveloped countries account for > 98 % of the malnourished people [1], which emphasises how asymmetrical this problem is. Deficiencies in metals such as iron and zinc [1], and vitamins such as A, B and C [1,2] are widespread in these nations and were linked to many diseases [3].

One way of fighting global hunger and micronutrient malnutrition is by producing food or dietary supplements, which are essential to correct inefficiencies in the human diet, allowing both to improve the health condition of chronic patients (*e.g.*, with diabetes [4] or kidney disease [5]) and to avoid the appearance of health issues, such as cancer and arteriosclerosis [2]. Moreover, nutrients such as vitamins, minerals and fatty acids and non-nutrients such as antioxidants have a known therapeutic potential against some viruses [6].

Among the most valuable biocompounds, antioxidants present particularly interesting properties, since they work as redox couples, ensuring a reduced state of animal cells and preventing or delaying oxidative damage by reactive oxidative species (ROS) [2,7]. Moreover, they help lowering the incidence of health issues such as arthritis and premature aging [3]. Even though synthetic antioxidants are still generally preferred at industrial scale due to their effectiveness and low price [8], the obtained bioactivity is lower than the one of their natural references, so there is an effort to find methods of extracting them from their natural sources without affecting their unique properties. Moreover, the interest in safe nutritional additives with immune boosting properties by consumers, especially during the coronavirus pandemic, has created the need of identifying natural and safe sources.

The most common feedstocks for natural antioxidants are fruits and vegetables [7,8], but using food as raw material raises moral issues and contributes to several environmental and social problems, such as larger farming areas, water scarceness, deforestation, higher food prices and even global hunger [9]. Therefore, the use of antioxidant-rich biowaste is a more feasible option, contributing simultaneously to a more circular economy and to a more sustainable production of therapeutics. Good examples of feedstock with these characteristics are fruit pomaces such as grape pomace, vegetable peals [7,10] and industrial wood wastes [11].

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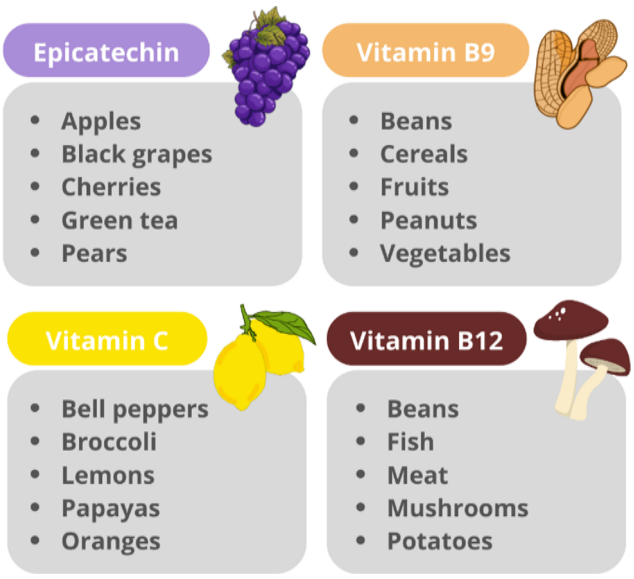

Fig. 1. Possible feedstocks for the extraction of epicatechin [16] and of vitamins B9 [19], B12 [22] and C [23].

Table 1
List of the chemicals used with respective chemical formula, suppliers, purities, CAS number and abbreviation.

Chemical	Supplier	Purity[a] / *m*%[b]	CAS	Abbreviation
Cyanocobalamin or vitamin B12 ($C_{63}H_{88}CoN_{14}O_{14}P$)	Sigma-Aldrich	> 98	68–19-9	B12
(-)-epicatechin ($C_{15}H_{14}O_6$)	Tokyo Chemical Industry	> 97	490–46-0	E
Ethanol (CH_3CH_2OH)	Sigma-Aldrich	> 99	64–17-5	–
(-)-ethyl L-lactate ($C_5H_{10}O_3$)	Sigma-Aldrich	> 98	97–64-3	EL
Folic acid or vitamin B9 ($C_{19}H_{19}N_7O_6$)	Sigma-Aldrich	> 97	59–30-3	B9
L-ascorbic acid or vitamin C ($C_6H_8O_6$)	Sigma-Aldrich	> 99	50–81-7	C
Purified water (H_2O)	VWR chemicals	–	7732–18-5	W
Sodium hydroxide (NaOH)	Merck	> 99	1310–73-2	–
Sodium citrate tribasic dihydrate ($C_6H_5Na_3O_7{\cdot}2H_2O$)	Sigma-Aldrich	> 99	6132–04-3	Na_3Citrate

[a] Provided by the supplier. [b] *m*% refers to mass percentage.

The main large-scale extraction process for antioxidants for biotechnological applications is the hydroalcoholic extraction. However, this method generally presents low product yield and purity, requiring high solvent consumption and, consequently, high operating costs and environmental impact [12]. Moreover, most biomolecules, such as proteins and enzymes, are not compatible with the alcohol-rich media [7]. On the other hand, an antioxidant-rich product can be obtained using green solvents, which are significantly less harmful for the environment than their alcoholic counterparts. A well-known green organic solvent is ethyl lactate, which is a hydrophilic, bio-renewable and biodegradable solvent with low toxicity towards humans and animals [13]. It can be obtained from corn fermentation and has been successfully applied in the extraction of biomolecules (vitamins, antioxidants and amino acids) in recent years [7,13–15], which can have particularly important roles in dietary supplements.

Epicatechin, a quite common flavonoid, is present in a wide variety of foods, such as chocolate and fruits (apples, black grapes, pears and cherries), and is one of the major constituents of green tea extract [16]. It has proved therapeutic potential in counteracting myelosuppression, reducing the pathophysiological complications of sickle cell anemia [16], and is considered a promising inhibitor of SARS-CoV-2 virus entry by disrupting interactions between host enzymes and the viral binding domain [17].

Vitamins B9 (folic acid) and B12 (cyanocobalamin) are active B-complex vitamins which are thought to significantly influence red blood and stem cells, tissue regeneration and the human immune system [18]. Vitamin B9 can be naturally found in meat, fermented dairy products, green leafy vegetables, cereals, fruits, beans and peanuts [19], and its antioxidant action has been reported to prevent skin aging, asthma and pneumonia [20]. Vitamin B12 is a cobalt-containing micronutrient that is synthesised by bacteria and archaea, and it is essential for the synthesis of deoxyribonucleic acid (DNA) and for energy production in cells [21]. It can be found in animal products, such as red meat (cow beef and lamb), fish (tuna and salmon), milk, eggs, beans, potatoes and in some mushrooms [22].

Vitamin C (ascorbic acid) is a particularly important vitamin for human nutrition, which is obtainable from vegetables (bell peppers, broccoli, cabbage) and from tropical (papaya, pineapple, mango) and citrus (orange, lemon, lime) fruits [23]. In recent years, the lack of this vitamin has been strongly related to accelerated aging, diabetes, leukaemia, ocular diseases and chronic inflammatory diseases [24].

Fig. 1 summarises some foods rich in the mentioned biomolecules. Waste from these foods may become the feedstock of future extractive processes of antioxidants with final application in dietary supplements.

Aqueous Biphasic Systems (ABS), also known as Aqueous Two-Phase Systems (ATPS), are a liquid–liquid extraction technique which provides biocompatible, non-toxic, non-flammable and low interfacial stress extractive media with a high content in water [25]. However, they present a disadvantage that is transversal to all kinds of ABS, from the classical ABS composed of polymer + polymer + water to the novel ones containing ionic liquids [26,27], deep eutectic solvents [28,29] and ethyl lactate [30], and which involves their generally low selectivity. Nevertheless, ABS are easy to scale up and to apply in continuous processes and have been commonly used in the extraction of proteins and of other labile biomolecules [25]. For example, a previous study of the research group [30] applied ethyl lactate-based ABS with organic salts (disodium tartrate, tripotassium citrate or trisodium citrate) to extract biomolecules commonly found in food waste (epicatechin, vitamin B12 or nicotinic acid) to set the ground for the sustainable production of value-added pharmaceutical and cosmetic products [30].

This work was focused on assessing the performance of a biodegradable Aqueous Biphasic System (ABS) containing a powerful green solvent (ethyl lactate) and an organic salt (trisodium citrate) in the extraction of chemical species with antioxidant properties, at 298.15 K and 0.1 MPa. For the first time, the extraction of epicatechin and vitamins B9, B12 and C was performed in this ABS, targeting the future valorisation of common biowastes, such as vegetable peals (*e.g.*, from potatoes) and fruit pomaces (*e.g.*, grape pomace).

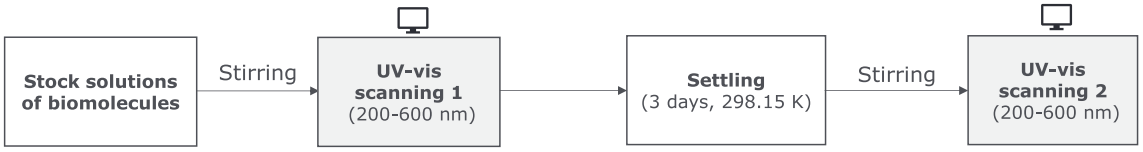

Fig. 2. Adopted procedure to evaluate the stability of the UV–vis absorbance spectra of the studied biomolecules.

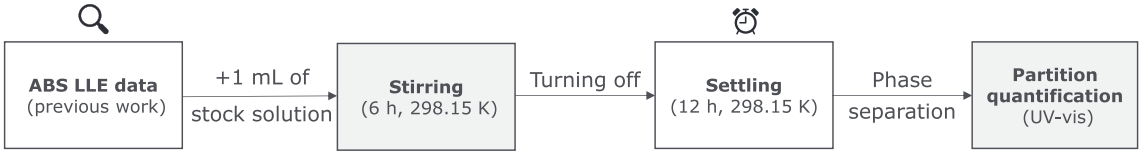

Fig. 3. Experimental procedure taken to perform the liquid–liquid extraction of epicatechin and vitamins B9, B12 and C in the ABS {ethyl lactate (1) + trisodium citrate (2) + water (3)} at 298.15 K and atmospheric pressure.

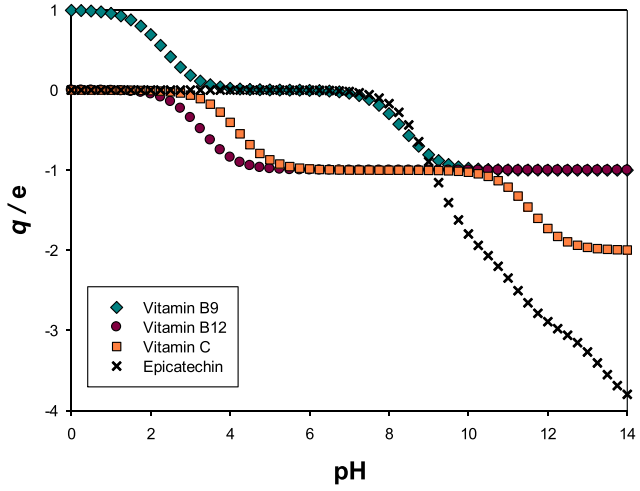

Fig. 4. Calculated mean electrical charge (q) for epicatechin and for vitamins B9, B12 and C, expressed in terms of the elementary charge (e), *i.e.*, 1.602 • 10^{-19}C.

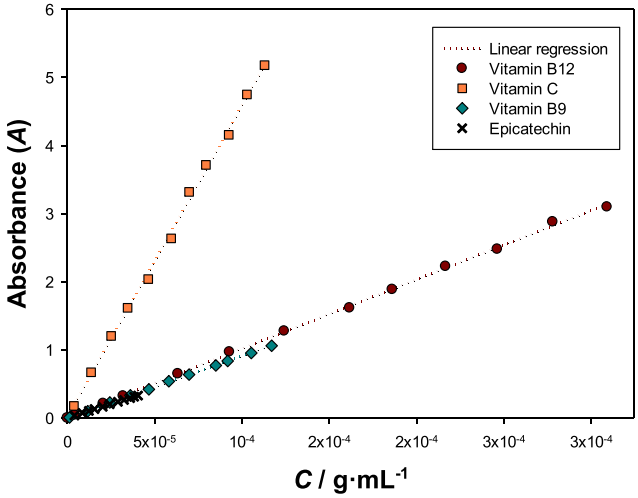

Fig. 5. Absorbance calibration curves at pH = 7.5 for epicatechin (278 nm), vitamin B9 (334 nm), vitamin B12 (363 nm) and vitamin C (266 nm). The first-degree fittings follow equations: $A = 8091\ 2 \cdot C_E$ g·mL $^{1}) + 0\ 0027$ with a determination coefficient (R^2) of 0.9998 [30], $A = 9101\ 9 \cdot C_{B9}$ g·mL $^{1}) + 0\ 0003$ with $R^2 = 0\ 9998$, $A = 10024 \cdot C_{B12}$ g·mL $^{1}) + 0\ 0123$ with $R^2 = 0\ 9998$ [30] and $A = 45935 \cdot C_C$ g·mL $^{1}) + 0\ 0432$ with $R^2 = 0\ 9996$, respectively.

2. Experimental

2.1. Chemicals

Table 1 summarises the chemicals used and the respective commercial suppliers, purities, Chemical Abstracts Service (CAS) number and abbreviation used in this work. None of the species required further purification.

2.2. Apparatus and experimental procedure

In the experimental determinations, mass (m) was determined with an ADAM AAA 250 L balance with measurement uncertainty of ± 10 ^{4} g, pH was assessed with a VWR pH 1100 L with measurement uncertainties of ± 0.001 in pH and ± 0.1 K in temperature. Moreover, density (ρ) was assessed using an Anton Paar DSA-5000 M densimeter, with measurement uncertainties of ± 3·10 ^{5} g·cm ^{3} in density and ± 0.01 K in temperature, while liquid volumes (V) were measured with an Eppendorf Multipipette E3x with a measurement uncertainty of ± 0.5 μL (when using the 200 μL tips). Temperature (T) was kept at 298.15 ± 0.01 K with an OVAN Therm H TH100E thermostatic and absorbance (A) was determined with a Thermo Scientific Varioskan Flash UV vis spectrophotometer with an uncertainty of ± 10^{-4}. When stirring was needed, samples were taken to a VWR vortex VV3 or to an IKA RO 10P magnetic stirrer.

2.2.1. Influence of system's pH in the UV–vis absorbance spectra

Stock solutions were prepared for epicatechin and for vitamins B9, B12 and C, with concentrations of about (15.4, 42.4, 31.2 and 7.60) · 10^{-5} g·mL ^{1}, respectively. When needed, the pH of the solutions was adjusted up to the pH of the ABS (with no biomolecules) by consecutively adding drops of a 0.5 M sodium hydroxide (NaOH) aqueous solution and mixing for 30 min in an IKA RO 10P magnetic stirrer, obtaining pH = 7.5. These values were determined with a VWR pH 1100 L pHmeter. The mass of added sodium hydroxide was assessed using the ADAM AAA 250 L balance and the antioxidant concentrations were recalculated having this in consideration.

Using an Eppendorf Multipipette E3x electronic pipette, 200 μL samples of each stock solution were taken and added to a Greiner bio-one polystyrene flat bottom plate with 96 wells and an absorbance scanning was performed from 200 to 600 nm with the Thermo Scientific Varioskan Flash UV vis spectrophotometer, after having stabilised the samples at 298.15 K.

The stock solutions were left settling for 3 days at 298.15 K without

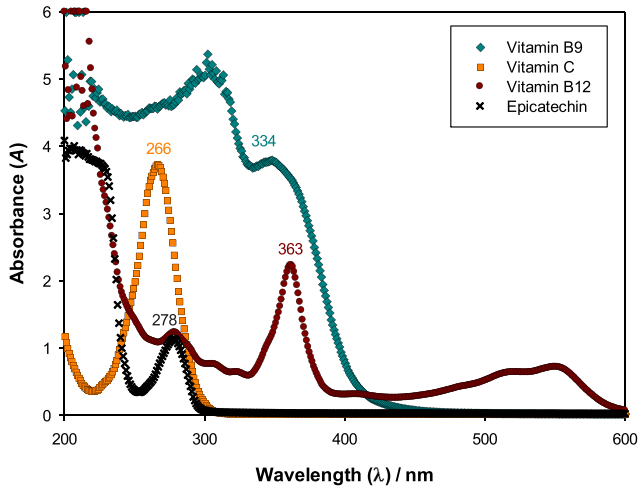

Fig. 6. UV–vis absorbance spectra at pH = 7.5, from 200 to 600 nm, for epicatechin, vitamin B9, vitamin B12, and vitamin C at (15.4, 42.4, 31.2 and 7.60) · 10^{-5} g·mL ^{1}, respectively.

Table 2
Determined tie-lines for the ABS {ethyl lactate (1) + trisodium citrate (2) + water (3)} at 298.15 K and 0.1 MPa [a,b] [15].

Tie-line	Feed		Phase	Separation		
	w_1/ *m*%	w_2/ *m*%		w_1/ *m*%	w_2/ *m*%	pH
1	30.0	11.0	Top	51.7	3.0	7.00
			Bottom	16.0	15.7	6.98
2	32.0	11.4	Top	57.5	2.0	6.98
			Bottom	12.3	18.5	6.96
3	34.3	11.7	Top	61.5	1.4	6.98
			Bottom	9.8	20.7	6.97
4	36.5	12.1	Top	65.0	1.0	7.00
			Bottom	7.9	23.0	7.00
5	38.5	12.3	Top	67.7	0.7	6.98
			Bottom	6.8	24.7	6.97
6	40.6	12.6	Top	70.1	0.5	6.98
			Bottom	5.5	26.6	7.00

[a] w_i stands for the mass percentage (*m*%) of species *i*.
[b] Standard uncertainties (u) are: $u(T) = 0.2$ K, $u(P) = 10$ kPa, $u(w_i) = 10^{-1}$ and u(pH) = 10 ^{2} [15].

any special protection from daylight, after which 200 μL samples were added to a new plate and an absorbance screening was conducted with the UV vis spectrophotometer following the same procedure. These two spectra were compared to evaluate the stability of the UV–vis absorbance spectra at the system's pH. The overall procedure is shown in Fig. 2.

2.2.2. UV–vis absorbance calibration curves

Mixtures with different concentrations of biomolecule and with a total volume of 2 mL were prepared in vials by diluting fresh stock solutions. The vials were capped, sealed with parafilm and vigorously stirred in a VWR VV3 vortex for about 1 min and in an IKA RO 10P magnetic stirrer for 10 min. Afterwards, 200 μL samples of each dilution were added to a Greiner bio-one polystyrene flat bottom plate with 96 wells and an absorbance scanning was carried out with the Thermo Scientific Varioskan Flash UV vis spectrophotometer, from 200 to 600 nm, with previous temperature stabilization at 298.15 K. Lastly, the UV vis absorbance calibration curves were prepared by plotting the biomolecules' concentrations and the respective UV vis absorbances at the chosen wavelengths and fitting the experimental points to a first-degree equation after having subtracted the absorbance of water (and plate) from the curve points. The chosen wavelengths were 278, 334, 363 and 266 nm for epicatechin and for vitamins B9, B12 and C, respectively.

Table 3
Experimental mass (m), absorbance (A) at chosen wavelength (λ), density (ρ), pH and mass loss (L_m) for the top and bottom phases in the extraction of epicatechin and vitamins B9, B12 and C using the ABS {ethyl lactate (1) + trisodium citrate (2) + water (3)} at 298.15 K and 0.1 MPa [b].

Tie-line	Phase	m/ g	L_m/ %	A	ρ / g·mL ^{1}	pH
		Epicatechin,λ = 278 nm				
1	Top	3.6786	0.87	0.7582	1.05573	7.541
	Bottom	6.4186		0.2325	1.12206	7.670
2	Top	4.4509	1.25	0.8049	1.05095	7.675
	Bottom	5.5871		0.1888	1.13730	7.625
3	Top	4.3920	1.92	0.8270	1.04645	7.656
	Bottom	5.4475		0.1705	1.14809	7.643
4	Top	4.6811	0.18	0.8495	1.04404	7.678
	Bottom	5.3176		0.1473	1.16507	7.704
5	Top	5.1993	2.18	0.8532	1.04604	7.756
	Bottom	4.6718		0.1395	1.17574	7.677
6	Top	5.2838	1.83	0.8726	1.04275	7.713
	Bottom	4.5601		0.1535	1.18907	7.788
		Vitamin B9,λ = 334 nm				
1	Top	3.5679	0.65	0.6160	1.05381	7.644
	Bottom	6.4195		0.5468	1.12229	7.534
2	Top	4.0928	0.87	0.6405	1.04788	7.748
	Bottom	5.8773		0.5117	1.13681	7.554
3	Top	4.5328	0.71	0.6468	1.04558	7.685
	Bottom	5.4912		0.5126	1.15010	7.551
4	Top	4.7552	0.66	0.6581	1.04354	7.735
	Bottom	5.2350		0.4960	1.16720	7.603
5	Top	4.9717	0.87	0.6580	1.04209	7.721
	Bottom	4.9846		0.4708	1.18528	7.621
6	Top	5.2187	0.63	0.6523	1.04118	7.851
	Bottom	4.8686		0.4556	1.19160	8.048
		Vitamin B12,λ = 363 nm				
1	Top	3.7499	0.48	2.6010	1.05619	7.487
	Bottom	6.2678		0.6602	1.12244	7.627
2	Top	4.1729	0.59	2.7220	1.04927	7.525
	Bottom	5.9126		0.4265	1.13519	7.639
3	Top	4.5510	0.65	2.6723	1.04610	7.770
	Bottom	5.4401		0.2717	1.15167	7.657
4	Top	4.8258	0.18	2.6360	1.04333	7.832
	Bottom	5.2352		0.1787	1.16632	7.663
5	Top	5.0746	0.54	2.5618	1.04238	7.883
	Bottom	4.8893		0.1333	1.17841	7.670
6	Top	5.3377	0.72	2.4767	1.04358	7.865
	Bottom	4.5932		0.0976	1.19212	7.953
		Vitamin C,λ = 266 nm				
1	Top	3.9504	0.62	1.7431	1.05456	7.840
	Bottom	6.0704		2.0115	1.12359	7.612
2	Top	4.3628	0.78	1.7191	1.05081	7.782
	Bottom	5.6416		2.0853	1.13733	7.660
3	Top	4.5833	1.46	1.6805	1.04557	7.814
	Bottom	5.3521		2.1733	1.15324	7.664
4	Top	4.8245	2.69	1.6733	1.04451	7.853
	Bottom	4.9874		2.3076	1.16629	7.699
5	Top	5.1483	1.42	1.6650	1.04147	7.959
	Bottom	4.7913		2.3838	1.17982	7.714
6	Top	5.2155	1.80	1.6595	1.04087	7.962
	Bottom	4.6855		2.5312	1.19489	7.831

[a] The measurement uncertainties (u) are: $u(m) = 10^{-4}$ g, $u(A) = 10^{-4}$, $u(\rho) = 3{\cdot}10^{-5}$ g·mL ^{1} and u(pH) = 10 ^{3}.

2.3. Liquid-liquid extraction of biomolecules

6 vials with mixtures of 10 mL were prepared corresponding to the tie-line compositions of the ABS {ethyl lactate (1) + trisodium citrate (2) + water (3)} determined in a previous work [15] by pipetting and weighing the pure compounds (water and ethyl lactate) and the aqueous solution of trisodium citrate salt (26.01 *m*%). In this preparation, 1 mL of the reported water-content in [15] was substituted by 1 mL of stock solution of the biomolecule being studied. Afterwards, the vials were

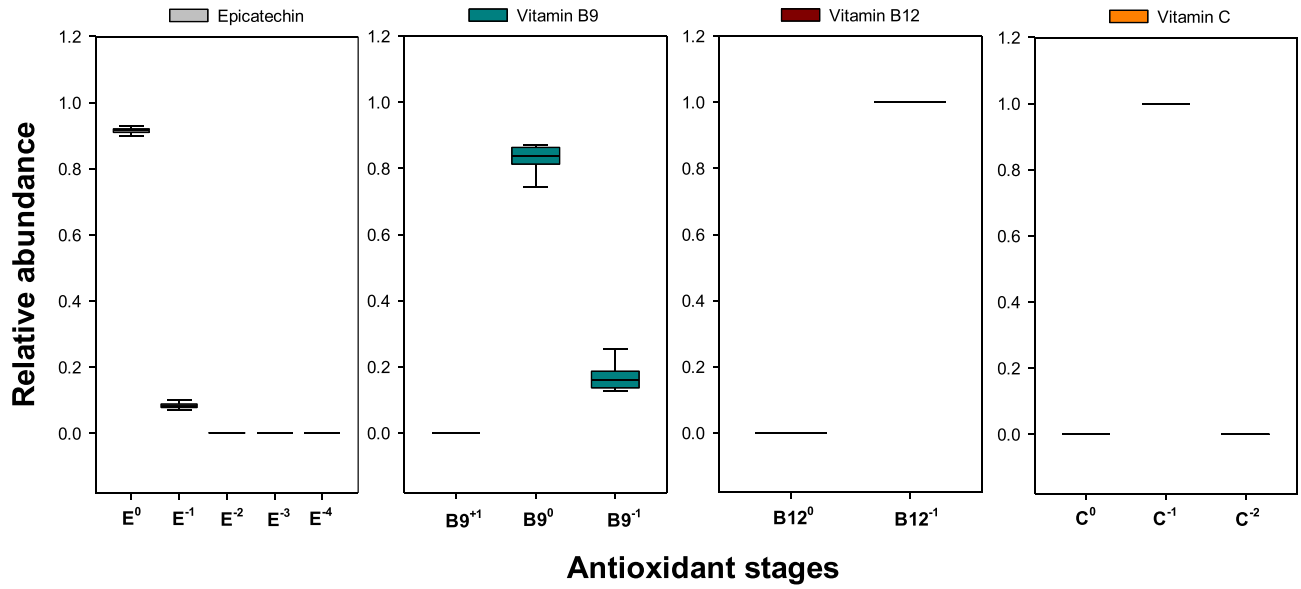

Fig. 7. Influence of the tie-line compositions in the relative abundance (fraction) of the biomolecule stages of epicatechin and of vitamins B9, B12 and C in the ABS {ethyl lactate (1) + trisodium citrate (2) + water (3)} at 298.15 K and 0.1 MPa. E^0, E^{-1}, E^{-2}, E^{-3} and E^{-4} stand for the biomolecule stages of epicatechin with electrical charges equal to 0, 1, 2, 3 and 4 e, respectively; $B9^{+1}$, $B9^0$ and $B9^{-1}$ stand for the biomolecule stages of folic acid with electrical charges equal to + 1, 0 and 1 e, respectively; $B12^0$ and $B12^{-1}$ stand for the biomolecule stages of cyanocobalamin with electrical charges equal to 0 and 1 e, respectively; C^0, C^{-1} and C^{-2} stand for the biomolecule stages of ascorbic acid with electrical charges equal to 0, 1 and 2 e, respectively, and e stands for the elementary charge ($1.602 \bullet 10^{-19}$C).

capped, sealed with parafilm and vigorously mixed in the vortex for 1 min and left in the OVAN Therm H TH100E thermostatic bath coupled with a magnetic stirrer for 6 h at a temperature of 298.15 K. After stirring, the vials were left settling overnight, which corresponds to approximately 12 h, at the same temperature, after which the top and bottom phases were carefully removed using the Eppendorf Multipipette E3x and weighed in the ADAM AAA 250 L balance. Next, the pH values of the two phases of each tie-line were assessed with the VWR pH 1100 L pHmeter and UV vis absorbances were determined using the spectrophotometer and following the methodology described above. Finally, liquid phase densities were determined with the Anton Paar DSA-5000 M densimeter and the instrument was cleaned between measurements with water and ethanol. The experimental procedure is summarised in Fig. 3.

3. Results and discussion

3.1. Influence of pH in the UV vis absorbance spectra

Antioxidants are very unstable molecules, especially when they present acidic properties, so they can possess differently charged chemical structures at certain pH values, which are known as biomolecule stages [7]. The presence of different electrical charges may affect, for example, the dielectric constant, the ionic strength, the viscosity and even the reactivity of the solution, for which solute partition between the phases might also be altered. Moreover, the UV–vis absorbance spectra of two successive biomolecule stages may be completely different, so understanding which species are present in the medium is essential to study liquid–liquid extraction of antioxidants in Aqueous Biphasic Systems (ABS) both to ensure a proper solute quantification and to adequately characterise the system. Further, one of the requirements of modern molecular simulations or thermodynamic modelling of partition, which may be applied in the future, is to know exactly which species are interacting.

Having in consideration the definition of the acid dissociation constant (K_a), and since the decimal logarithms of the dissociation constants (pK_a) of epicatechin (8.72, 9.49, 11.23 and 13.80 [31]), vitamin B9 (2.35 and 8.38 [32]), vitamin B12 (3.28 [33]) and vitamin C (4.17 and 11.57 [34]) are known, the ratios between two successive biomolecule stages, *i.e.*, between a biomolecule of a certain electrical charge and its closest reduced state, can be determined as function of the liquid phase's pH using equation (1) [7].

$$\frac{\left[A^{q_0\ (i\ 1)}\right]}{\left[A^{q_0\ i}\right]} = 10^{pH_{phase}\ pK_a^i} \tag{1}$$

where q_0 is the electrical charge of the biomolecule at pH = 0, i is the number of the dissociation constant (pK_a^i) being considered, pH_{phase} is the pH of the liquid phase and $\left[A^{q_0\ (i\ 1)}\right]$ and $\left[A^{q_0\ i}\right]$ are the molar concentrations of the biomolecule stages with electrical charges of $q_0\ (i\ 1)$ and $q_0\ i$, respectively.

Then, the fraction or relative abundance of the less reduced biomolecule stage in the reaction with pK_a^i, *i.e.*, with electrical charge equal to $q_0\ (i\ 1)$, can be determined by:

$$x_{A^{q_0\ (i\ 1)}} = \frac{\left[A^{q_0\ (i\ 1)}\right]}{\sum_{j=1}^{i_{max}+1}\left[A^{q_0\ (j\ 1)}\right]} \tag{2}$$

where i_{max} is the maximum number of hydrogens that the biomolecule under study can donate, *i.e.*, it is the number of different pK_a for each biomolecule.

Dividing the numerator and the denominator of the right-hand term of equation (2) by $\left[A^{q_0\ 1}\right]$ comes:

$$x_{A^{q_0\ (i\ 1)}} = \frac{\frac{\left[A^{q_0\ (i\ 1)}\right]}{\left[A^{q_0\ 1}\right]}}{\frac{\sum_{j=1}^{i_{max}+1}\left[A^{q_0\ (j\ 1)}\right]}{\left[A^{q_0\ 1}\right]}} \tag{3}$$

Finally, to know the relative abundance of each biomolecule stage, equation (3) can be rearranged using the ratios determined by equation (1) into:

$$x_{A^{q_0\ (i\ 1)}} = \frac{\frac{\left[A^{q_0\ (i\ 1)}\right]}{\left[A^{q_0\ 1}\right]}}{\left(\frac{\left[A^{q_0}\right]}{\left[A^{q_0\ 1}\right]} + \frac{\left[A^{q_0\ 1}\right]}{\left[A^{q_0\ 1}\right]} + \sum_{j=2}^{i_{max}}\left[\prod_{k=2}^{j}\frac{\left[A^{q_0\ k}\right]}{\left[A^{q_0\ (k\ 1)}\right]}\right]\right)} \tag{4}$$

Table 4
Calculated biomolecule's concentration (C) for each phase, partition coefficients (K) for each tie-line and tie-line lengths (TLL) for the extraction of epicatechin and vitamins B9, B12 and C in the ABS {ethyl lactate (1) + trisodium citrate (2) + water (3)} at 298.15 K and 0.1 MPa.

Tie-line	Phase	C / g·mL^{-1}	K	TLL / % [15]
		Epicatechin		
1	Top	$3.17 \cdot 10^{-5}$	3.83 ± 0.01	37.85
	Bottom	$8.28 \cdot 10^{-6}$		
2	Top	$2.94 \cdot 10^{-5}$	4.35 ± 0.01	48.18
	Bottom	$6.76 \cdot 10^{-6}$		
3	Top	$3.08 \cdot 10^{-5}$	4.61 ± 0.01	55.17
	Bottom	$6.68 \cdot 10^{-6}$		
4	Top	$2.97 \cdot 10^{-5}$	4.73 ± 0.01	61.17
	Bottom	$6.28 \cdot 10^{-6}$		
5	Top	$2.78 \cdot 10^{-5}$	5.27 ± 0.01	65.44
	Bottom	$5.27 \cdot 10^{-6}$		
6	Top	$2.75 \cdot 10^{-5}$	5.44 ± 0.01	69.68
	Bottom	$5.05 \cdot 10^{-6}$		
		Vitamin B9		
1	Top	$6.17 \cdot 10^{-5}$	1.12 ± 0.02	37.85
	Bottom	$5.50 \cdot 10^{-5}$		
2	Top	$6.50 \cdot 10^{-5}$	1.27 ± 0.02	48.18
	Bottom	$5.11 \cdot 10^{-5}$		
3	Top	$6.60 \cdot 10^{-5}$	1.29 ± 0.02	55.17
	Bottom	$5.11 \cdot 10^{-5}$		
4	Top	$6.70 \cdot 10^{-5}$	1.36 ± 0.02	61.17
	Bottom	$4.93 \cdot 10^{-5}$		
5	Top	$6.68 \cdot 10^{-5}$	1.45 ± 0.02	65.44
	Bottom	$4.61 \cdot 10^{-5}$		
6	Top	$6.60 \cdot 10^{-5}$	1.48 ± 0.02	69.68
	Bottom	$4.45 \cdot 10^{-5}$		
		Vitamin B12		
1	Top	$2.54 \cdot 10^{-4}$	4.17 ± 0.03	37.85
	Bottom	$6.09 \cdot 10^{-5}$		
2	Top	$2.67 \cdot 10^{-4}$	7.11 ± 0.04	48.18
	Bottom	$3.75 \cdot 10^{-5}$		
3	Top	$2.62 \cdot 10^{-4}$	11.87 ± 0.08	55.17
	Bottom	$2.21 \cdot 10^{-5}$		
4	Top	$2.58 \cdot 10^{-4}$	20.30 ± 0.13	61.17
	Bottom	$1.27 \cdot 10^{-5}$		
5	Top	$2.50 \cdot 10^{-4}$	31.80 ± 0.21	65.44
	Bottom	$7.88 \cdot 10^{-6}$		
6	Top	$2.42 \cdot 10^{-4}$	55.44 ± 0.38	69.68
	Bottom	$4.36 \cdot 10^{-6}$		
		Vitamin C		
1	Top	$2.67 \cdot 10^{-5}$	0.70 ± 0.04	37.85
	Bottom	$4.00 \cdot 10^{-5}$		
2	Top	$2.47 \cdot 10^{-5}$	0.62 ± 0.05	48.18
	Bottom	$4.22 \cdot 10^{-5}$		
3	Top	$2.36 \cdot 10^{-5}$	0.57 ± 0.05	55.17
	Bottom	$4.44 \cdot 10^{-5}$		
4	Top	$2.27 \cdot 10^{-5}$	0.51 ± 0.05	61.17
	Bottom	$4.77 \cdot 10^{-5}$		
5	Top	$2.20 \cdot 10^{-5}$	0.48 ± 0.05	65.44
	Bottom	$4.93 \cdot 10^{-5}$		
6	Top	$2.13 \cdot 10^{-5}$	0.44 ± 0.05	69.68
	Bottom	$5.13 \cdot 10^{-5}$		

which is the same as:

$$x_{A^{q_0}\ (i\ 1)} = \frac{\frac{[A^{q_0}\ (i\ 1)]}{[A^{q_0}\ 1]}}{\left(\frac{[A^{q_0}]}{[A^{q_0}\ 1]} + 1 + \sum_{j=2}^{i_{max}} \left[\prod_{k=2}^{j} \frac{[A^{q_0}\ k]}{[A^{q_0}\ (k\ 1)]}\right]\right)} \quad (5)$$

This way, Eq 5 allows to determine the fractions or relative abundance of all biomolecule stages using the ratios calculated with Eq 1. Then, the mean electrical charge of the antioxidant in solution (q) can be determined by a weighted arithmetic mean:

$$q = \sum_{i=1}^{i_{max}} [x_{A^{q_0}\ (i\ 1)} \cdot (q_0 \quad (i \quad 1))] + \left(1 \quad \sum_{i=1}^{i_{max}} x_{A^{q_0}\ (i\ 1)}\right) \cdot \left(q_0 \quad i_{max}\right) \quad (6)$$

Fig. 1 shows the calculated mean electrical charges (q) at different pH values for epicatechin and for vitamins B9, B12 and C. In the Appendices, Tables A1–A4, the calculated mole fractions of each biomolecule stage for the data points plotted in Fig. 4 can be observed.

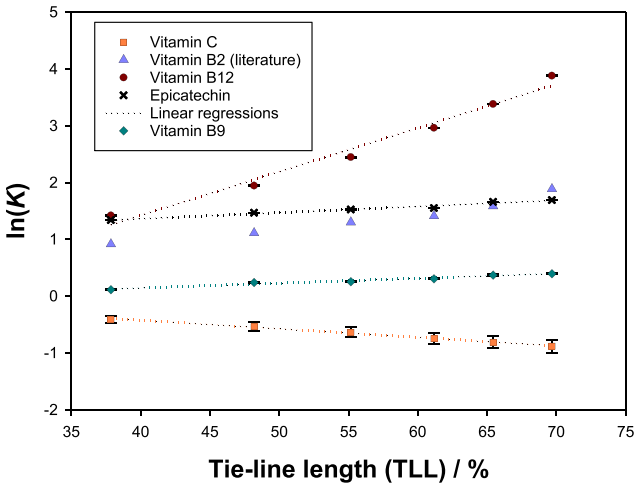

Fig. 8. Relation of the tie-line length (TLL) with the natural logarithm of the experimental partition coefficients (K) in the ABS {ethyl lactate (1) + trisodium citrate (2) + water (3)} at 298.15 K and 0.1 MPa for epicatechin and for vitamins B9, B12, C (this work) and B2 (from [7]).

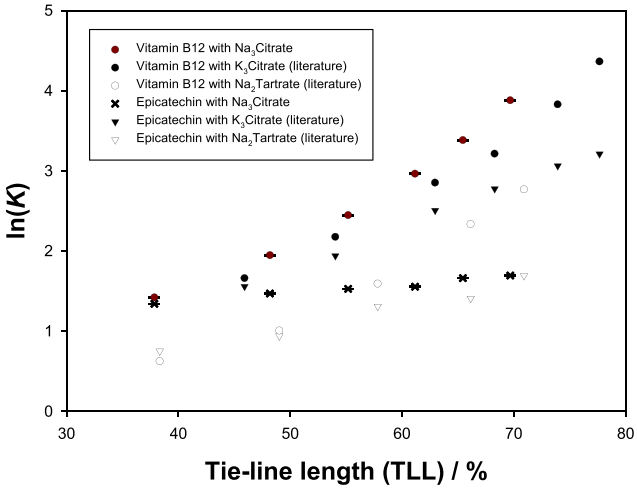

Fig. 9. Relation of the tie-line length (TLL) with the natural logarithm of the experimental partition coefficients (K) in the ABS {ethyl lactate (1) + Na_3Citrate (this work) or K_3Citrate [30] or Na_2Tartrate [30] (2) + water (3)} at 298.15 K and 0.1 MPa for vitamin B12 and epicatechin.

Previous works [7,15] reported a pH of about 7.5 for the ternary phases of the ABS {ethyl lactate (1) + trisodium citrate (2) + water (3)} at 298.15 K and atmospheric pressure. As it can be seen in Fig. 4, q is heavily dependent on pH, so different charges can coexist in the system. However, at this pH, the studied biomolecules show an integer or close to integer mean electrical charge (q), which means that they are almost entirely present in that biomolecule stage, as can be confirmed in Tables A1-A4, in the Appendices, by their relative abundances: x_{E^0} = 0 94, x_{B9^0} = 0 88, $x_{B12\ 1}$ = 1 00 and $x_{C\ 1}$ = 1 00.

3.2. UV–vis absorbance calibration curves

After having determined which species were present at the system's pH (≃ 7.5), the UV–visible absorbance calibration curves were carried out at that pH value, as Fig. 5 shows, so as to properly quantify the biomolecules after phase separation. In the determinations, the upper boundaries of the concentrations were defined by the maximum

Table 5
Calculated liquid phase volumes (V), solute losses (L_s), extraction efficiency (E) intervals and literature-based tie-line lengths (TLL) for the extraction of epicatechin and vitamins B9, B12 and C in the ABS {ethyl lactate (1) + trisodium citrate (2) + water (3)} at 298.15 K and 0.1 MPa.

Tie-line	V / mL	L_s / %	E / %	TLL / % [15]
	Epicatechin			
1	3.484	3.92	(67.2 – 71.2) ± 0.4	37.85
2	5.720	3.43	(76.2 – 79.7) ± 0.5	48.18
3	4.235	1.59	(79.0 – 80.6) ± 0.5	55.17
4	4.913	1.52	(81.0 – 81.6) ± 0.5	61.17
5	4.197	3.16	(83.4 – 82.6) ± 0.5	65.44
6	4.745	3.46	(84.8 – 88.2) ± 0.5	69.68
	Vitamin B9			
1	3.386	2.53	(38.9 – 41.4) ± 0.1	37.85
2	5.720	3.42	(47.4 – 50.8) ± 0.1	48.18
3	3.906	1.58	(53.1 – 54.7) ± 0.1	55.17
4	5.170	2.51	(56.6 – 59.1) ± 0.1	61.17
5	4.335	4.52	(59.4 – 63.9) ± 0.1	65.44
6	4.775	4.52	(61.6 – 66.2) ± 0.1	69.68
	Vitamin B12			
1	3.550	0.68	(72.1 – 72.8) ± 0.1	37.85
2	5.584	0.01	(84.5 – 84.5) ± 0.1	48.18
3	3.977	1.10	(90.6 – 91.7) ± 0.1	55.17
4	5.208	0.99	(94.5 – 95.5) ± 0.1	61.17
5	4.350	0.09	(97.5 – 97.4) ± 0.1	65.44
6	4.724	0.20	(98.5 – 98.7) ± 0.1	69.68
	Vitamin C			
1	3.746	4.64	(30.1 – 34.8) ± 0.1	37.85
2	5.403	6.03	(30.9 – 36.9) ± 0.1	48.18
3	4.152	6.75	(31.1 – 37.9) ± 0.1	55.17
4	4.960	6.96	(31.5 – 38.5) ± 0.1	61.17
5	4.384	6.95	(32.7 – 39.7) ± 0.1	65.44
6	4.641	7.02	(32.2 – 39.2) ± 0.1	69.68

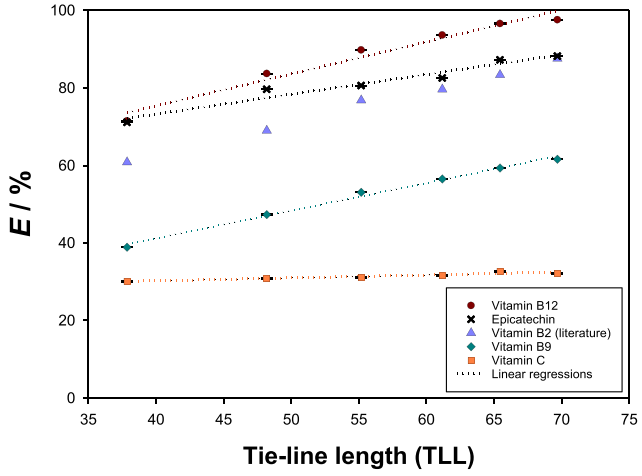

Fig. 10. Relation of the tie-line length (TLL) with the extraction efficiencies (E) in the ABS {ethyl lactate (1) + trisodium citrate (2) + water (3)} at 298.15 K and 0.1 MPa for epicatechin and for vitamins B9, B12, C (this work) and B2 (from [7]).

solubility in water of the species and by the useful absorbance range of the spectrophotometer. The absorbances of water (and plate) were subtracted and the calibration curves were determined at the wavelengths of local or global maxima in which the other ABS species (ethyl lactate and trisodium citrate) and pH adjuster (sodium hydroxide) did not interfere [7]. The UV–vis absorbance spectra of the studied biomolecules are shown in Fig. 6 and the respective data points can be observed in Tables A5-A11, in the Appendices.

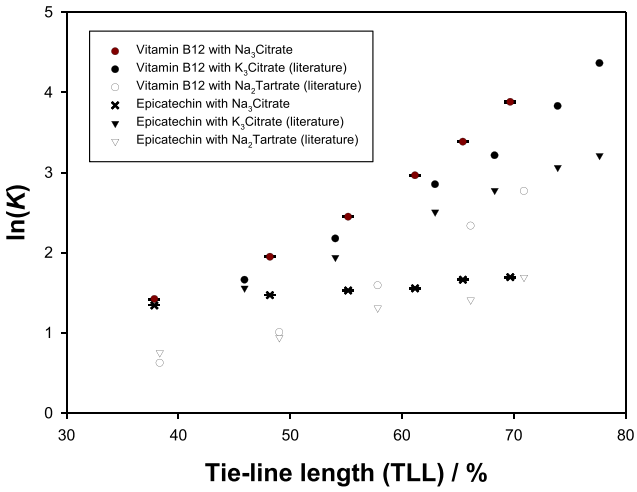

Fig. 11. Relation of the tie-line length (TLL) with the extraction efficiencies (E) in the ABS {ethyl lactate (1) + Na_3Citrate (this work) or K_3Citrate [30] or Na_2Tartrate [30] (2) + water (3)} at 298.15 K and 0.1 MPa for vitamin B12 and epicatechin.

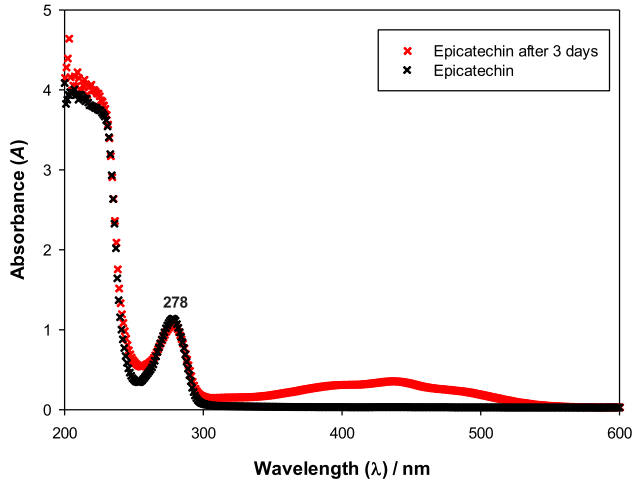

Fig. A1. UV–vis absorbance spectra of the aqueous stock solution of epicatechin ($1.54{\cdot}10^{-4}$ g·mL ^{1} and pH = 7.5) in the moment of preparation and after 72 h of settling at 298.15 K and 0.1 MPa.

3.3. Partitioning of biomolecules

The ABS {ethyl lactate (1) + trisodium citrate (2) + water (3)} was the focus of a previous work [15], so its liquid–liquid equilibria (LLE) data were not determined in this study and partition was performed in the reported tie-lines, which can be observed in Table 2.

The extractions of the commercial biomolecules (epicatechin and vitamins B9, B12 and C) were performed in this ABS at 298.15 K and 0.1 MPa following the experimental procedure explained in section 2.3. After the equilibrium was reached, mass (m), absorbance (A), pH and density (ρ) were measured for the top and bottom phases. Then, the mass losses (L_m) were calculated, in percentage, using:

$$L_m = \frac{m_2 \quad m_1}{m_1} \cdot 100 \tag{7}$$

where m_1 is the total mass (feed) and m_2 is the sum of masses of the two phases after equilibrium was reached.

As Table 3 shows, the phase separations for the studied systems were very similar. Moreover, absorbances for top phases were always higher

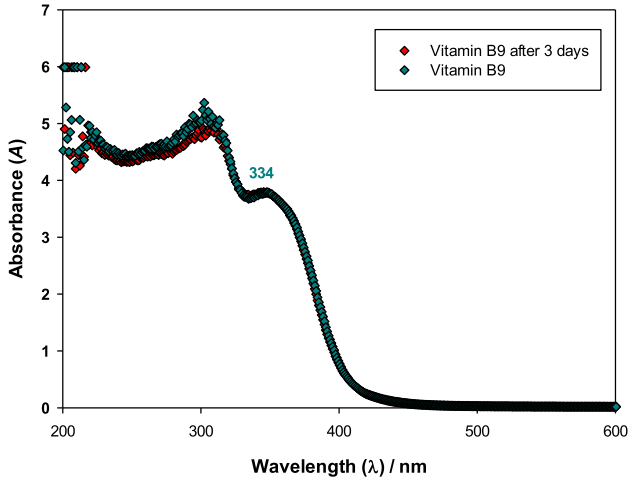

Fig. A2. UV–vis absorbance spectra of the aqueous stock solution of vitamin B9 ($4.24{\cdot}10^{-4}$ g·mL ^{1} and pH = 7.5) in the moment of preparation and after 72 h of settling at 298.15 K and 0.1 MPa.

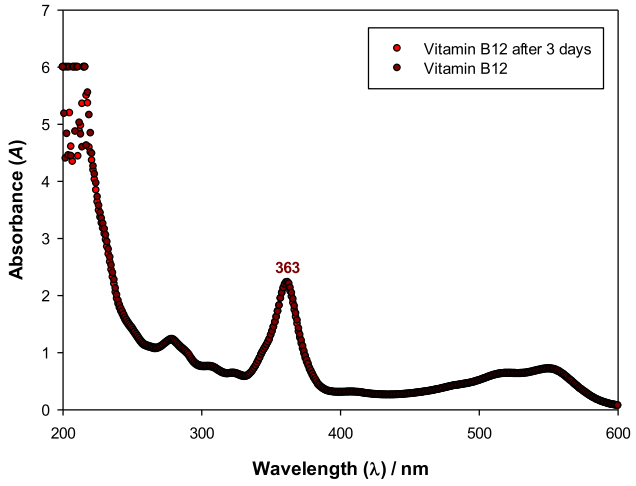

Fig. A3. UV–vis absorbance spectra of the aqueous stock solution of vitamin B12 ($3.12{\cdot}10^{-4}$ g·mL ^{1} and pH = 7.5) in the moment of preparation and after 72 h of settling at 298.15 K and 0.1 MPa.

than for the bottom ones in ABS with epicatechin, vitamin B9 and vitamin B12. However, for vitamin C, the opposite was verified, which hints that this biomolecule preferentially migrates for the water-rich phase. Ethyl lactate, which is the extraction solvent, is more abundantly present in the top phases and apparently enhances these phases' affinity for epicatechin, vitamin B9 and vitamin B12.

As expected, the verified pH values for the two liquid phases of each tie-line in each system were alike, which implies similar distribution of antioxidant charges and mean electrical charge (q) in both phases. This way, the same calibration curve can be applied for top and bottom phases. Furthermore, even though pH did not vary significantly between different tie-line compositions of the same system, some variations were found in the relative abundance (fraction) of the biomolecule stages of the studied biomolecules, as Fig. 7 shows.

In Fig. 7, it can be observed that the distribution of electrical charges in vitamins B12 and C is not affected by using different tie-lines, while epicatechin presented some variation in the compositions of neutral and mononegative species. Vitamin B9, on the other hand, was significantly more affected by using different tie-line compositions since it has a pK_a near the system's pH.

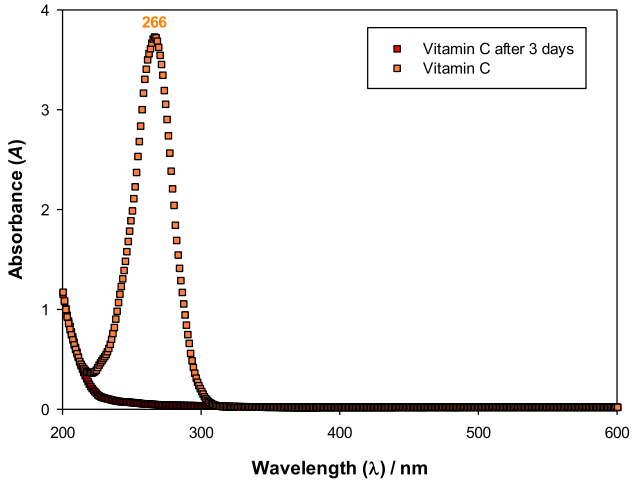

Fig. A4. UV–vis absorbance spectra of the aqueous stock solution of vitamin C ($7.60{\cdot}10^{-5}$ g·mL ^{1} and pH = 7.5) in the moment of preparation and after 72 h of settling at 298.15 K and 0.1 MPa.

After having determined the biomolecules' concentrations using the UV–vis absorbance calibration curves, the partition coefficients (K) were calculated according to their definition:

$$K_i = \frac{\overset{\text{top}}{i}}{\underset{\text{bottom}}{i}} \tag{8}$$

where i is the tie-line number and C_i^{top} and C_i^{bottom} correspond to the biomolecule's concentration in the top and bottom phases, respectively.

The calculated biomolecule concentrations and partition coefficients can be observed in Table 4, together with the tie-line lengths reported in a previous work [15].

As Table 4 shows, vitamin B12 presented the largest partition coefficients, which means that this biomolecule more significantly migrates to the top phases. On the other hand, vitamin C was the only one with partition coefficients below unity, so the only one in which the top phases' concentrations of biomolecule were smaller than the ones of the bottom phases.

In this work, a larger tie-line number (from 1 to 6, as Table 2 shows) corresponded to a larger length of tie-line (TLL), *i.e.*, to a more distinct phase composition. If the species preferentially diffuse to a water-rich medium, an increasing TLL will cause a reduction in the values of the partition coefficients. As Fig. 8 illustrates, all biomolecules except vitamin C suffered an increase in the top phase concentration for longer tie-lines, so it can be said that the ethyl lactate-rich phase has more affinity for these species.

In Fig. 8, the more positive the slope of the straight lines found, the more favoured solute migration for the top phase is. Therefore, it can be concluded, once again, that the ethyl lactate-rich phase has more affinity for vitamin B12 and epicatechin, and less for vitamins B9 and C. The obtained results were compared with data for another B-complex vitamin, riboflavin (vitamin B2), from a previous work [7], and it can be noticed that, even though it presented a lower natural logarithm of K than epicatechin for the first tie-line, its partition coefficient became larger than the one of epicatechin for the longest tie-line, since it is more positively affected by an increase in the tie-line length (TLL).

Fig. 9 compares the partition coefficients obtained in this work for cyanocobalamin (vitamin B12) and epicatechin with the extractions carried out in the ABS {ethyl lactate (1) + tripotassium citrate (K_3Citrate) or disodium tartrate (Na_2Tartrate) (2) + water} at 298.15 K and 0.1 MPa in a previous work of the research group [30]. Generally, organic salts based on the citrate ion provided better extractions than the tartrate-based ones. Moreover, the largest partition coefficients for

Table A1
Calculated fractions of each biomolecule stage and mean antioxidant charge (q) at different pH values for epicatechin, expressed in terms of the elementary charge (e), *i.e.*, $1.602 \bullet 10^{-19}$C.

pH	x_{E^0} [a]	$x_{E\ 1}$	$x_{E\ 2}$	$x_{E\ 3}$	$x_{E\ 4}$	q / e
0.00	1.00	0.00	0.00	0.00	0.00	0.00
0.25	1.00	0.00	0.00	0.00	0.00	0.00
0.50	1.00	0.00	0.00	0.00	0.00	0.00
0.75	1.00	0.00	0.00	0.00	0.00	0.00
1.00	1.00	0.00	0.00	0.00	0.00	0.00
1.25	1.00	0.00	0.00	0.00	0.00	0.00
1.50	1.00	0.00	0.00	0.00	0.00	0.00
1.75	1.00	0.00	0.00	0.00	0.00	0.00
2.00	1.00	0.00	0.00	0.00	0.00	0.00
2.25	1.00	0.00	0.00	0.00	0.00	0.00
2.50	1.00	0.00	0.00	0.00	0.00	0.00
2.75	1.00	0.00	0.00	0.00	0.00	0.00
3.00	1.00	0.00	0.00	0.00	0.00	0.00
3.25	1.00	0.00	0.00	0.00	0.00	0.00
3.50	1.00	0.00	0.00	0.00	0.00	0.00
3.75	1.00	0.00	0.00	0.00	0.00	0.00
4.00	1.00	0.00	0.00	0.00	0.00	0.00
4.25	1.00	0.00	0.00	0.00	0.00	0.00
4.50	1.00	0.00	0.00	0.00	0.00	0.00
4.75	1.00	0.00	0.00	0.00	0.00	0.00
5.00	1.00	0.00	0.00	0.00	0.00	0.00
5.25	1.00	0.00	0.00	0.00	0.00	0.00
5.50	1.00	0.00	0.00	0.00	0.00	0.00
5.75	1.00	0.00	0.00	0.00	0.00	0.00
6.00	1.00	0.00	0.00	0.00	0.00	0.00
6.25	1.00	0.00	0.00	0.00	0.00	0.00
6.50	0.99	0.01	0.00	0.00	0.00	0.01
6.75	0.99	0.01	0.00	0.00	0.00	0.01
7.00	0.98	0.02	0.00	0.00	0.00	0.02
7.25	0.97	0.03	0.00	0.00	0.00	0.03
7.50	0.94	0.06	0.00	0.00	0.00	0.06
7.75	0.90	0.10	0.00	0.00	0.00	0.10
8.00	0.84	0.16	0.01	0.00	0.00	0.17
8.25	0.74	0.25	0.01	0.00	0.00	0.28
8.50	0.60	0.36	0.04	0.00	0.00	0.44
8.75	0.44	0.47	0.09	0.00	0.00	0.65
9.00	0.28	0.54	0.17	0.00	0.00	0.89
9.25	0.16	0.53	0.31	0.00	0.00	1.16
9.50	0.08	0.45	0.46	0.01	0.00	1.41
9.75	0.03	0.34	0.61	0.02	0.00	1.62
10.00	0.01	0.22	0.72	0.04	0.00	1.80
10.25	0.00	0.14	0.78	0.08	0.00	1.94
10.50	0.00	0.08	0.78	0.14	0.00	2.07
10.75	0.00	0.04	0.72	0.24	0.00	2.20
11.00	0.00	0.02	0.62	0.36	0.00	2.35
11.25	0.00	0.01	0.48	0.51	0.00	2.50
11.50	0.00	0.00	0.35	0.64	0.01	2.66
11.75	0.00	0.00	0.23	0.75	0.02	2.79
12.00	0.00	0.00	0.14	0.83	0.03	2.89
12.25	0.00	0.00	0.08	0.86	0.06	2.98
12.50	0.00	0.00	0.05	0.85	0.11	3.06
12.75	0.00	0.00	0.02	0.80	0.18	3.15
13.00	0.00	0.00	0.01	0.71	0.28	3.27
13.25	0.00	0.00	0.01	0.58	0.41	3.41
13.50	0.00	0.00	0.00	0.44	0.56	3.55
13.75	0.00	0.00	0.00	0.31	0.69	3.69
14.00	0.00	0.00	0.00	0.20	0.80	3.80

[a] x_{E^0}, $x_{E\ 1}$, $x_{E\ 2}$, $x_{E\ 3}$ and $x_{E\ 4}$ are the fractions of epicatechin in the biomolecule stages of electrical charges equal to 0, 1, 2, 3 and 4 e, respectively.

Table A2
Calculated fractions of each biomolecule stage and mean antioxidant charge (q) at different pH values for vitamin B9, expressed in terms of the elementary charge (e), *i.e.*, $1.602 \bullet 10^{-19}$C.

pH	$x_{B9^{+1}}$ [a]	x_{B9^0}	$x_{B9\ 1}$	q / e
0.00	1.00	0.00	0.00	1.00
0.25	0.99	0.01	0.00	0.99
0.50	0.99	0.01	0.00	0.99
0.75	0.98	0.02	0.00	0.98
1.00	0.96	0.04	0.00	0.96
1.25	0.93	0.07	0.00	0.93
1.50	0.88	0.12	0.00	0.88
1.75	0.80	0.20	0.00	0.80
2.00	0.69	0.31	0.00	0.69
2.25	0.56	0.44	0.00	0.56
2.50	0.41	0.59	0.00	0.41
2.75	0.28	0.72	0.00	0.28
3.00	0.18	0.82	0.00	0.18
3.25	0.11	0.89	0.00	0.11
3.50	0.07	0.93	0.00	0.07
3.75	0.04	0.96	0.00	0.04
4.00	0.02	0.98	0.00	0.02
4.25	0.01	0.99	0.00	0.01
4.50	0.01	0.99	0.00	0.01
4.75	0.00	1.00	0.00	0.00
5.00	0.00	1.00	0.00	0.00
5.25	0.00	1.00	0.00	0.00
5.50	0.00	1.00	0.00	0.00
5.75	0.00	1.00	0.00	0.00
6.00	0.00	1.00	0.00	0.00
6.25	0.00	0.99	0.01	0.01
6.50	0.00	0.99	0.01	0.01
6.75	0.00	0.98	0.02	0.02
7.00	0.00	0.96	0.04	0.04
7.25	0.00	0.93	0.07	0.07
7.50	0.00	0.88	0.12	0.12
7.75	0.00	0.81	0.19	0.19
8.00	0.00	0.71	0.29	0.29
8.25	0.00	0.57	0.43	0.43
8.50	0.00	0.43	0.57	0.57
8.75	0.00	0.30	0.70	0.70
9.00	0.00	0.19	0.81	0.81
9.25	0.00	0.12	0.88	0.88
9.50	0.00	0.07	0.93	0.93
9.75	0.00	0.04	0.96	0.96
10.00	0.00	0.02	0.98	0.98
10.25	0.00	0.01	0.99	0.99
10.50	0.00	0.01	0.99	0.99
10.75	0.00	0.00	1.00	1.00
11.00	0.00	0.00	1.00	1.00
11.25	0.00	0.00	1.00	1.00
11.50	0.00	0.00	1.00	1.00
11.75	0.00	0.00	1.00	1.00
12.00	0.00	0.00	1.00	1.00
12.25	0.00	0.00	1.00	1.00
12.50	0.00	0.00	1.00	1.00
12.75	0.00	0.00	1.00	1.00
13.00	0.00	0.00	1.00	1.00
13.25	0.00	0.00	1.00	1.00
13.50	0.00	0.00	1.00	1.00
13.75	0.00	0.00	1.00	1.00
14.00	0.00	0.00	1.00	1.00

[a] $x_{B9^{+1}}$, x_{B9^0}, and $x_{B9\ 1}$ are the fractions of vitamin B9 in the biomolecule stages of electrical charges equal to 0, 1 and 2 e, respectively.

vitamin B12 were observed in the ABS composed of trisodium citrate (Na_3Citrate), while epicatechin's extraction was more favoured by the ABS with K_3Citrate.

3.4. Mass balance

After having determined the partition coefficients, it is important to validate the analytical method by performing a mass balance for the biomolecules under study, *i.e.*, verifying that all the added solute is being considered by the analytical method. To do so, first, the liquid phase volumes (V) were determined by:

$$V_j = \frac{m_j}{\rho_j} \tag{9}$$

where V_j is the liquid phase volume, m_j is the measured mass and ρ_j is the measured density for phase j.

Then, the mass balance was checked by calculating the solute losses (L_s) using equation (10).

Table A3
Calculated fractions of each biomolecule stage and mean antioxidant charge (q) at different pH values for vitamin B12, expressed in terms of the elementary charge (e), *i.e.*, $1.602 \bullet 10^{-19}$C.

pH	x_{B12^0} [a]	x_{B12^1}	q / e
0.00	1.00	0.00	0.00
0.25	1.00	0.00	0.00
0.50	1.00	0.00	0.00
0.75	1.00	0.00	0.00
1.00	0.99	0.01	0.01
1.25	0.99	0.01	0.01
1.50	0.98	0.02	0.02
1.75	0.97	0.03	0.03
2.00	0.95	0.05	0.05
2.25	0.91	0.09	0.09
2.50	0.86	0.14	0.14
2.75	0.77	0.23	0.23
3.00	0.66	0.34	0.34
3.25	0.52	0.48	0.48
3.50	0.38	0.62	0.62
3.75	0.25	0.75	0.75
4.00	0.16	0.84	0.84
4.25	0.10	0.90	0.90
4.50	0.06	0.94	0.94
4.75	0.03	0.97	0.97
5.00	0.02	0.98	0.98
5.25	0.01	0.99	0.99
5.50	0.01	0.99	0.99
5.75	0.00	1.00	1.00
6.00	0.00	1.00	1.00
6.25	0.00	1.00	1.00
6.50	0.00	1.00	1.00
6.75	0.00	1.00	1.00
7.00	0.00	1.00	1.00
7.25	0.00	1.00	1.00
7.50	0.00	1.00	1.00
7.75	0.00	1.00	1.00
8.00	0.00	1.00	1.00
8.25	0.00	1.00	1.00
8.50	0.00	1.00	1.00
8.75	0.00	1.00	1.00
9.00	0.00	1.00	1.00
9.25	0.00	1.00	1.00
9.50	0.00	1.00	1.00
9.75	0.00	1.00	1.00
10.00	0.00	1.00	1.00
10.25	0.00	1.00	1.00
10.50	0.00	1.00	1.00
10.75	0.00	1.00	1.00
11.00	0.00	1.00	1.00
11.25	0.00	1.00	1.00
11.50	0.00	1.00	1.00
11.75	0.00	1.00	1.00
12.00	0.00	1.00	1.00
12.25	0.00	1.00	1.00
12.50	0.00	1.00	1.00
12.75	0.00	1.00	1.00
13.00	0.00	1.00	1.00
13.25	0.00	1.00	1.00
13.50	0.00	1.00	1.00
13.75	0.00	1.00	1.00
14.00	0.00	1.00	1.00

[a] x_{B12^0}, and x_{B12^1} are the fractions of vitamin B12 in the biomolecule stages of electrical charges equal to 0 and 1 e, respectively.

Table A4
Calculated fractions of each biomolecule stage and mean antioxidant charge (q) at different pH values for vitamin C, expressed in terms of the elementary charge (e), *i.e.*, $1.602 \bullet 10^{-19}$C.

pH	x_{C^0} [a]	x_{C^1}	x_{C^2}	q / e
0.00	1.00	0.00	0.00	0.00
0.25	1.00	0.00	0.00	0.00
0.50	1.00	0.00	0.00	0.00
0.75	1.00	0.00	0.00	0.00
1.00	1.00	0.00	0.00	0.00
1.25	1.00	0.00	0.00	0.00
1.50	1.00	0.00	0.00	0.00
1.75	1.00	0.00	0.00	0.00
2.00	0.99	0.01	0.00	0.01
2.25	0.99	0.01	0.00	0.01
2.50	0.98	0.02	0.00	0.02
2.75	0.96	0.04	0.00	0.04
3.00	0.94	0.06	0.00	0.06
3.25	0.89	0.11	0.00	0.11
3.50	0.82	0.18	0.00	0.18
3.75	0.72	0.28	0.00	0.28
4.00	0.60	0.40	0.00	0.40
4.25	0.45	0.55	0.00	0.55
4.50	0.32	0.68	0.00	0.68
4.75	0.21	0.79	0.00	0.79
5.00	0.13	0.87	0.00	0.87
5.25	0.08	0.92	0.00	0.92
5.50	0.04	0.96	0.00	0.96
5.75	0.03	0.97	0.00	0.97
6.00	0.01	0.99	0.00	0.99
6.25	0.01	0.99	0.00	0.99
6.50	0.00	1.00	0.00	1.00
6.75	0.00	1.00	0.00	1.00
7.00	0.00	1.00	0.00	1.00
7.25	0.00	1.00	0.00	1.00
7.50	0.00	1.00	0.00	1.00
7.75	0.00	1.00	0.00	1.00
8.00	0.00	1.00	0.00	1.00
8.25	0.00	1.00	0.00	1.00
8.50	0.00	1.00	0.00	1.00
8.75	0.00	1.00	0.00	1.00
9.00	0.00	1.00	0.00	1.00
9.25	0.00	1.00	0.00	1.00
9.50	0.00	0.99	0.01	1.01
9.75	0.00	0.99	0.01	1.01
10.00	0.00	0.97	0.03	1.03
10.25	0.00	0.95	0.05	1.05
10.50	0.00	0.92	0.08	1.08
10.75	0.00	0.87	0.13	1.13
11.00	0.00	0.79	0.21	1.21
11.25	0.00	0.68	0.32	1.32
11.50	0.00	0.54	0.46	1.46
11.75	0.00	0.40	0.60	1.60
12.00	0.00	0.27	0.73	1.73
12.25	0.00	0.17	0.83	1.83
12.50	0.00	0.11	0.89	1.89
12.75	0.00	0.06	0.94	1.94
13.00	0.00	0.04	0.96	1.96
13.25	0.00	0.02	0.98	1.98
13.50	0.00	0.01	0.99	1.99
13.75	0.00	0.01	0.99	1.99
14.00	0.00	0.00	1.00	2.00

[a] x_{C^0}, x_{C^1}, and x_{C^2} are the fractions of vitamin C in the biomolecule stages of electrical charges equal to 0, 1 and 2 e, respectively.

$$L_s \Big/ \% = \frac{m_{s2} \quad m_{s1}}{m_{s1}} \cdot 100 \tag{10}$$

where m_{s1} is the added mass of antioxidant (present in 1 mL of stock solution, as described in section 2.3) and m_{s2} is the quantified experimental mass of antioxidant, which was calculated using equation (11).

$$m_{s2} = V_i^{top} \; {}_i^{top} + V_i^{bottom} \; {}_i^{bottom} \tag{11}$$

where V_i^{top} and V_i^{bottom} are the calculated experimental volumes of the top and bottom phases, respectively, and i refers to the tie-line number.

Moreover, after having calculated the mass of biomolecule in each phase, the extraction efficiencies of each tie-line (E) were determined using equation (12).

$$E = \frac{m_{s2}}{m_{s1}} \cdot 100 \tag{12}$$

Since the solute losses quantified using equation (10) may or not be

Table A5
Measured UV–vis absorbance from 200 to 600 nm at pH ≃ 7.5 for aqueous solutions of epicatechin (E) and vitamins B9, B12 and C at (15.4, 42.4, 31.2 and 7.60) · 10^{-5} g·mL ^{1}, respectively [a].

Wavelength / nm	E	B9	B12	C
200	4.0863	4.5354	6.0000	1.1782
201	3.8253	6.0000	5.1825	1.0907
202	3.8778	5.2928	4.4016	1.0057
203	3.9361	4.7379	4.8325	0.9298
204	3.9716	4.5144	4.4536	0.8642
205	3.9606	4.8571	6.0000	0.8055
206	3.9665	5.0729	4.4415	0.7515
207	4.0052	6.0000	6.0000	0.7006
208	3.9339	6.0000	6.0000	0.6513
209	3.9427	4.3169	4.8715	0.6049
210	3.8772	6.0000	6.0000	0.5618
211	3.9428	4.5180	6.0000	0.5237
212	3.9340	5.0747	5.0273	0.4835
213	3.9064	6.0000	4.8214	0.4562
214	3.8925	4.5844	4.5956	0.4331
215	3.8579	4.3711	6.0000	0.4137
216	3.8915	4.6257	6.0000	0.3974
217	3.8417	4.6029	4.6262	0.3845
218	3.8079	4.9753	5.5522	0.3750
219	3.8069	4.9687	5.1591	0.3696
220	3.7961	4.7544	4.8432	0.3680
221	3.7887	4.8631	4.4839	0.3700
222	3.7729	4.7269	4.2619	0.3755
223	3.7758	4.8025	4.1216	0.3847
224	3.7551	4.8562	3.9661	0.3984
225	3.7455	4.6670	3.7251	0.4243
226	3.7425	4.7029	3.5726	0.4485
227	3.7148	4.7103	3.4455	0.4718
228	3.6894	4.6368	3.3481	0.4931
229	3.6672	4.6367	3.2569	0.5126
230	3.6223	4.5782	3.1615	0.5324
231	3.5427	4.6038	3.0574	0.5546
232	3.4079	4.5915	2.9377	0.5816
233	3.1976	4.5244	2.8071	0.6146
234	2.9318	4.5635	2.6676	0.6568
235	2.6351	4.5327	2.5281	0.7076
236	2.3261	4.5219	2.3965	0.7650
237	2.0192	4.5459	2.2734	0.8245
238	1.6437	4.4934	2.1241	0.9089
239	1.3703	4.4624	2.0084	0.9877
240	1.1564	4.4913	1.9085	1.0745
241	1.0007	4.4648	1.8316	1.1581
242	0.8794	4.4421	1.7713	1.2354
243	0.7717	4.4668	1.7175	1.3130
244	0.6715	4.4520	1.6644	1.3964
245	0.5858	4.4803	1.6154	1.4873
246	0.5168	4.4356	1.5710	1.5847
247	0.4637	4.4372	1.5330	1.6862
248	0.4237	4.4513	1.4992	1.7901
249	0.3944	4.4492	1.4674	1.8940
250	0.3745	4.4546	1.4369	1.9920
251	0.3584	4.4385	1.3977	2.1152
252	0.3495	4.4418	1.3631	2.2339
253	0.3445	4.4854	1.3264	2.3776
254	0.3452	4.4833	1.2889	2.5373
255	0.3524	4.5105	1.2539	2.6866
256	0.3671	4.4764	1.2190	2.8420
257	0.3877	4.5099	1.1875	3.0063
258	0.4105	4.5291	1.1618	3.1715
259	0.4324	4.5341	1.1440	3.3112
260	0.4531	4.5768	1.1319	3.4113
261	0.4783	4.5734	1.1212	3.5054
262	0.5012	4.5686	1.1139	3.5655

[a] The measurement uncertainties (u) are: $u(\mathrm{A}) = 10^{-4}$ and $u(\mathrm{pH}) = 0\,001$.

Table A6
Measured UV–vis absorbance from 200 to 600 nm at pH ≃ 7.5 for aqueous solutions of epicatechin (E) and vitamins B9, B12 and C at (15.4, 42.4, 31.2 and 7.60) · 10^{-5} g·mL ^{1}, respectively [a] - continuation.

Wavelength / nm	E	B9	B12	C
263	0.5294	4.5708	1.1064	3.6167
264	0.5664	4.6041	1.0986	3.6718
265	0.6121	4.5997	1.0911	3.7139
266	0.6670	4.5633	1.0847	3.7340
267	0.7230	4.5977	1.0819	3.7260
268	0.7763	4.6090	1.0840	3.6919
269	0.8275	4.6326	1.0905	3.6302
270	0.8774	4.6491	1.1010	3.5501
271	0.9239	4.6536	1.1146	3.4583
272	0.9681	4.6215	1.1308	3.3509
273	1.0178	4.6403	1.1533	3.1973
274	1.0530	4.6086	1.1728	3.0596
275	1.0840	4.6961	1.1922	2.9078
276	1.1107	4.6006	1.2107	2.7437
277	1.1305	4.6186	1.2259	2.5718
278	1.1393	4.6289	1.2350	2.3910
279	1.1331	4.6723	1.2353	2.2132
280	1.1128	4.7316	1.2266	2.0472
281	1.0699	4.6935	1.2048	1.8491
282	1.0239	4.8160	1.1813	1.6965
283	0.9694	4.7774	1.1549	1.5508
284	0.9097	4.8026	1.1293	1.4180
285	0.8444	4.8367	1.1055	1.2945
286	0.7708	4.8432	1.0837	1.1757
287	0.6880	4.7882	1.0630	1.0604
288	0.5998	4.9421	1.0420	0.9496
289	0.5137	4.8561	1.0199	0.8483
290	0.4323	4.9151	0.9950	0.7540
291	0.3562	5.0054	0.9658	0.6654
292	0.2750	4.9934	0.9252	0.5649
293	0.2209	4.9453	0.8913	0.4912
294	0.1774	5.1473	0.8590	0.4250
295	0.1440	4.9979	0.8310	0.3670
296	0.1193	5.0181	0.8084	0.3181
297	0.1017	5.0695	0.7914	0.2769
298	0.0895	5.0411	0.7794	0.2417
299	0.0810	5.0296	0.7716	0.2115
300	0.0744	5.0627	0.7665	0.1837
301	0.0693	5.2524	0.7637	0.1584
302	0.0642	5.3754	0.7628	0.1305
303	0.0610	5.1549	0.7634	0.1115
304	0.0583	5.0700	0.7646	0.0953
305	0.0562	5.2209	0.7653	0.0821
306	0.0542	5.0923	0.7646	0.0710
307	0.0525	5.0053	0.7614	0.0621
308	0.0511	5.1279	0.7552	0.0549
309	0.0500	4.9611	0.7460	0.0492
310	0.0489	4.9356	0.7345	0.0450
311	0.0479	5.0056	0.7174	0.0410
312	0.0473	4.9868	0.7023	0.0386
313	0.0467	5.0680	0.6871	0.0366
314	0.0462	4.7824	0.6725	0.0349
315	0.0458	4.8226	0.6596	0.0337
316	0.0454	4.8047	0.6492	0.0326
317	0.0450	4.7103	0.6421	0.0318
318	0.0446	4.6647	0.6384	0.0309
319	0.0442	4.5324	0.6380	0.0303
320	0.0439	4.4100	0.6400	0.0298
321	0.0435	4.3399	0.6426	0.0294
322	0.0432	4.2216	0.6446	0.0291
323	0.0429	4.1307	0.6446	0.0288
324	0.0428	4.0465	0.6422	0.0286
325	0.0426	3.9781	0.6372	0.0284

[a] The measurement uncertainties (u) are: $u(\mathrm{A}) = 10^{-4}$ and $u(\mathrm{pH}) = 0\,001$.

in the top phase, an extraction efficiency interval can be found using the values determined for E as minimum boundary and summing L_s with E for the maximum boundary.

The results for the calculated liquid phase volumes (V), solute losses (L_s) and extraction efficiency (E) intervals are presented in Table 5.

As observed in Table 5, low solute losses were obtained for epicatechin (< 4 %), vitamin B9 (< 5 %) and vitamin B12 (generally < 1 %). However, the magnitude of solute losses was more preponderant for vitamin C, which reached losses of 7 %. As Figs. A1-A4in the Appendices show, the used global or local maxima of the UV–vis absorbance spectra

Table A7
Measured UV–vis absorbance from 200 to 600 nm at pH ≃ 7.5 for aqueous solutions of epicatechin (E) and vitamins B9, B12 and C at (15.4, 42.4, 31.2 and 7.60) · 10^{-5} g·mL ^{1}, respectively [a] - continuation.

Wavelength / nm	E	B9	B12	C
326	0.0424	3.9371	0.6298	0.0283
327	0.0422	3.8522	0.6185	0.0281
328	0.0420	3.8408	0.6086	0.0280
329	0.0418	3.7858	0.5993	0.0278
330	0.0414	3.7417	0.5920	0.0277
331	0.0412	3.7215	0.5884	0.0276
332	0.0409	3.7209	0.5898	0.0274
333	0.0406	3.7085	0.5966	0.0273
334	0.0404	3.6865	0.6093	0.0271
335	0.0402	3.6932	0.6327	0.0269
336	0.0399	3.7019	0.6579	0.0269
337	0.0396	3.7169	0.6881	0.0268
338	0.0395	3.7096	0.7239	0.0267
339	0.0394	3.7320	0.7639	0.0266
340	0.0390	3.7480	0.8081	0.0264
341	0.0388	3.7462	0.8549	0.0263
342	0.0385	3.7711	0.9040	0.0263
343	0.0382	3.7746	0.9640	0.0261
344	0.0381	3.7729	1.0100	0.0261
345	0.0379	3.7774	1.0538	0.0260
346	0.0376	3.7866	1.0958	0.0259
347	0.0375	3.7965	1.1384	0.0257
348	0.0373	3.8007	1.1832	0.0256
349	0.0371	3.7807	1.2329	0.0256
350	0.0368	3.7735	1.3040	0.0255
351	0.0365	3.7662	1.3708	0.0253
352	0.0362	3.7336	1.4463	0.0253
353	0.0360	3.7158	1.5298	0.0250
354	0.0357	3.7046	1.6221	0.0249
355	0.0355	3.6783	1.7207	0.0248
356	0.0353	3.6625	1.8235	0.0247
357	0.0351	3.6317	1.9515	0.0245
358	0.0347	3.6072	2.0466	0.0244
359	0.0345	3.5853	2.1312	0.0242
360	0.0345	3.5543	2.1932	0.0241
361	0.0342	3.5323	2.2293	0.0240
362	0.0341	3.5071	2.2341	0.0239
363	0.0340	3.4685	2.2074	0.0239
364	0.0338	3.4282	2.1321	0.0238
365	0.0337	3.3958	2.0431	0.0238
366	0.0336	3.3457	1.9349	0.0237
367	0.0335	3.2973	1.8133	0.0236
368	0.0332	3.2418	1.6873	0.0235
369	0.0330	3.1848	1.5638	0.0233
370	0.0329	3.1113	1.4210	0.0232
371	0.0329	3.0434	1.3113	0.0233
372	0.0327	2.9769	1.2093	0.0232
373	0.0326	2.9036	1.1118	0.0233
374	0.0325	2.8273	1.0209	0.0233
375	0.0326	2.7524	0.9395	0.0233
376	0.0324	2.6762	0.8668	0.0233
377	0.0326	2.6014	0.8026	0.0234
378	0.0325	2.5063	0.7307	0.0235
379	0.0325	2.4257	0.6783	0.0236
380	0.0325	2.3403	0.6282	0.0237
381	0.0324	2.2503	0.5809	0.0236
382	0.0324	2.1582	0.5372	0.0237
383	0.0323	2.0659	0.4978	0.0237
384	0.0323	1.9538	0.4565	0.0238
385	0.0322	1.8670	0.4291	0.0238
386	0.0322	1.7833	0.4062	0.0239
387	0.0322	1.6985	0.3866	0.0239
388	0.0320	1.6137	0.3699	0.0240

[a] The measurement uncertainties (u) are: $u(\mathrm{A}) = 10^{-4}$ and $u(\mathrm{pH}) = 0\,001$.

Table A8
Measured UV–vis absorbance from 200 to 600 nm at pH ≃ 7.5 for aqueous solutions of epicatechin (E) and vitamins B9, B12 and C at (15.4, 42.4, 31.2 and 7.60) · 10^{-5} g·mL ^{1}, respectively [a] - continuation.

Wavelength / nm	E	B9	B12	C
389	0.0321	1.5300	0.3557	0.0240
390	0.0319	1.4277	0.3414	0.0240
391	0.0319	1.3516	0.3326	0.0241
392	0.0318	1.2799	0.3258	0.0241
393	0.0317	1.2125	0.3206	0.0242
394	0.0316	1.1484	0.3169	0.0242
395	0.0316	1.0861	0.3141	0.0243
396	0.0316	1.0250	0.3120	0.0243
397	0.0315	0.9496	0.3103	0.0243
398	0.0314	0.8922	0.3096	0.0244
399	0.0314	0.8368	0.3093	0.0243
400	0.0313	0.7842	0.3093	0.0243
401	0.0312	0.7344	0.3097	0.0243
402	0.0311	0.6783	0.3104	0.0243
403	0.0310	0.6373	0.3111	0.0244
404	0.0311	0.6002	0.3118	0.0245
405	0.0310	0.5664	0.3126	0.0244
406	0.0310	0.5350	0.3133	0.0244
407	0.0310	0.4983	0.3139	0.0245
408	0.0310	0.4703	0.3139	0.0244
409	0.0310	0.4438	0.3137	0.0246
410	0.0310	0.4191	0.3132	0.0246
411	0.0310	0.3962	0.3120	0.0245
412	0.0310	0.3752	0.3104	0.0246
413	0.0310	0.3553	0.3082	0.0246
414	0.0309	0.3321	0.3049	0.0246
415	0.0309	0.3146	0.3016	0.0245
416	0.0309	0.2985	0.2983	0.0246
417	0.0309	0.2837	0.2948	0.0246
418	0.0309	0.2674	0.2903	0.0245
419	0.0309	0.2558	0.2871	0.0246
420	0.0310	0.2452	0.2840	0.0245
421	0.0310	0.2355	0.2812	0.0246
422	0.0311	0.2267	0.2784	0.0246
423	0.0311	0.2182	0.2760	0.0246
424	0.0311	0.2103	0.2739	0.0247
425	0.0312	0.2010	0.2713	0.0247
426	0.0313	0.1939	0.2694	0.0249
427	0.0314	0.1869	0.2679	0.0247
428	0.0314	0.1800	0.2664	0.0247
429	0.0313	0.1717	0.2648	0.0248
430	0.0314	0.1650	0.2636	0.0247
431	0.0314	0.1587	0.2628	0.0247
432	0.0315	0.1528	0.2622	0.0248
433	0.0315	0.1474	0.2616	0.0247
434	0.0315	0.1422	0.2615	0.0248
435	0.0315	0.1361	0.2614	0.0248
436	0.0315	0.1314	0.2616	0.0248
437	0.0314	0.1269	0.2619	0.0248
438	0.0314	0.1224	0.2624	0.0248
439	0.0314	0.1170	0.2633	0.0249
440	0.0313	0.1133	0.2641	0.0248
441	0.0314	0.1098	0.2651	0.0249
442	0.0315	0.1062	0.2663	0.0249
443	0.0313	0.1028	0.2678	0.0249
444	0.0313	0.0995	0.2692	0.0249
445	0.0312	0.0955	0.2713	0.0249
446	0.0312	0.0926	0.2732	0.0248
447	0.0312	0.0898	0.2752	0.0249
448	0.0311	0.0873	0.2771	0.0248
449	0.0310	0.0849	0.2792	0.0249
450	0.0310	0.0819	0.2819	0.0249
451	0.0309	0.0795	0.2844	0.0248

[a]The measurement uncertainties (u) are: $u(\mathrm{A}) = 10^{-4}$ and $u(\mathrm{pH}) = 0\,001$.

of epicatechin and of vitamins B9 and B12 and their intensity were preserved even after 3 days of settling, but the same cannot be said about vitamin C. So, this apparent solute loss may be due to oxidation of this very reactive antioxidant and/or hydrolysis. Nevertheless, since low solute losses were generally obtained for the studied biomolecules, the validity of the analytical method was assured, confirming the reported partition coefficients (K) and extraction efficiencies (E).

Table 5 also shows high extraction efficiencies (E) for vitamin B12 and epicatechin, with tie-line 6 compositions yielding the best result, while vitamins B9 and C presented less promising results. E increased

Table A9
Measured UV–vis absorbance from 200 to 600 nm at pH ≃ 7.5 for aqueous solutions of epicatechin (E) and vitamins B9, B12 and C at (15.4, 42.4, 31.2 and 7.60) · 10^{-5} g·mL ^{1}, respectively [a] - continuation.

Wavelength / nm	E	B9	B12	C
452	0.0309	0.0773	0.2870	0.0249
453	0.0308	0.0752	0.2897	0.0250
454	0.0307	0.0727	0.2931	0.0249
455	0.0307	0.0708	0.2960	0.0248
456	0.0306	0.0688	0.2991	0.0249
457	0.0304	0.0669	0.3022	0.0248
458	0.0305	0.0653	0.3054	0.0249
459	0.0303	0.0639	0.3084	0.0249
460	0.0303	0.0623	0.3122	0.0248
461	0.0304	0.0613	0.3153	0.0248
462	0.0304	0.0600	0.3187	0.0250
463	0.0303	0.0587	0.3232	0.0249
464	0.0303	0.0576	0.3271	0.0250
465	0.0303	0.0563	0.3315	0.0250
466	0.0302	0.0553	0.3359	0.0249
467	0.0301	0.0542	0.3404	0.0250
468	0.0300	0.0531	0.3461	0.0249
469	0.0300	0.0523	0.3509	0.0249
470	0.0301	0.0515	0.3558	0.0248
471	0.0300	0.0508	0.3610	0.0250
472	0.0300	0.0499	0.3663	0.0249
473	0.0299	0.0491	0.3729	0.0250
474	0.0300	0.0484	0.3783	0.0249
475	0.0299	0.0477	0.3838	0.0250
476	0.0297	0.0471	0.3892	0.0250
477	0.0298	0.0464	0.3958	0.0248
478	0.0297	0.0457	0.4010	0.0249
479	0.0296	0.0452	0.4065	0.0249
480	0.0296	0.0445	0.4120	0.0249
481	0.0295	0.0439	0.4184	0.0248
482	0.0295	0.0435	0.4230	0.0248
483	0.0295	0.0432	0.4270	0.0249
484	0.0295	0.0428	0.4306	0.0248
485	0.0295	0.0425	0.4340	0.0248
486	0.0294	0.0420	0.4382	0.0249
487	0.0296	0.0418	0.4418	0.0250
488	0.0295	0.0416	0.4457	0.0250
489	0.0294	0.0410	0.4508	0.0249
490	0.0294	0.0408	0.4548	0.0250
491	0.0294	0.0405	0.4589	0.0251
492	0.0294	0.0402	0.4632	0.0250
493	0.0293	0.0398	0.4676	0.0250
494	0.0292	0.0396	0.4739	0.0251
495	0.0292	0.0392	0.4795	0.0250
496	0.0292	0.0389	0.4857	0.0251
497	0.0292	0.0387	0.4924	0.0250
498	0.0290	0.0383	0.5007	0.0250
499	0.0290	0.0381	0.5076	0.0251
500	0.0289	0.0379	0.5149	0.0250
501	0.0289	0.0376	0.5226	0.0251
502	0.0288	0.0373	0.5329	0.0250
503	0.0288	0.0371	0.5414	0.0250
504	0.0288	0.0368	0.5502	0.0250
505	0.0287	0.0367	0.5590	0.0250
506	0.0286	0.0364	0.5699	0.0249
507	0.0287	0.0363	0.5785	0.0249
508	0.0285	0.0360	0.5867	0.0250
509	0.0285	0.0360	0.5947	0.0249
510	0.0284	0.0357	0.6038	0.0250
511	0.0284	0.0356	0.6105	0.0250
512	0.0283	0.0353	0.6166	0.0250
513	0.0284	0.0353	0.6222	0.0251
514	0.0283	0.0350	0.6283	0.0250

[a]The measurement uncertainties (u) are: $u(A) = 10^{4}$ and $u(pH) = 0\,001$.

Table A10
Measured UV–vis absorbance from 200 to 600 nm at pH ≃ 7.5 for aqueous solutions of epicatechin (E) and vitamins B9, B12 and C at (15.4, 42.4, 31.2 and 7.60) · 10^{-5} g·mL ^{1}, respectively [a] - continuation.

Wavelength / nm	E	B9	B12	C
515	0.0284	0.0349	0.6327	0.0250
516	0.0282	0.0347	0.6362	0.0250
517	0.0282	0.0347	0.6390	0.0250
518	0.0281	0.0345	0.6414	0.0249
519	0.0280	0.0343	0.6426	0.0250
520	0.0280	0.0342	0.6433	0.0251
521	0.0280	0.0340	0.6435	0.0250
522	0.0280	0.0339	0.6433	0.0251
523	0.0278	0.0339	0.6426	0.0251
524	0.0277	0.0336	0.6419	0.0249
525	0.0277	0.0334	0.6409	0.0249
526	0.0276	0.0334	0.6402	0.0249
527	0.0276	0.0332	0.6397	0.0250
528	0.0276	0.0332	0.6396	0.0249
529	0.0276	0.0330	0.6399	0.0249
530	0.0276	0.0329	0.6405	0.0249
531	0.0276	0.0328	0.6416	0.0251
532	0.0276	0.0327	0.6436	0.0251
533	0.0277	0.0327	0.6458	0.0251
534	0.0276	0.0325	0.6485	0.0250
535	0.0275	0.0323	0.6516	0.0251
536	0.0274	0.0321	0.6563	0.0250
537	0.0273	0.0321	0.6609	0.0251
538	0.0274	0.0320	0.6663	0.0250
539	0.0273	0.0318	0.6735	0.0249
540	0.0273	0.0316	0.6793	0.0249
541	0.0272	0.0316	0.6848	0.0249
542	0.0272	0.0315	0.6913	0.0249
543	0.0273	0.0313	0.6963	0.0250
544	0.0272	0.0313	0.7012	0.0251
545	0.0273	0.0312	0.7061	0.0251
546	0.0273	0.0313	0.7117	0.0250
547	0.0272	0.0309	0.7157	0.0251
548	0.0273	0.0310	0.7190	0.0250
549	0.0271	0.0308	0.7216	0.0250
550	0.0272	0.0306	0.7224	0.0250
551	0.0271	0.0306	0.7219	0.0250
552	0.0271	0.0304	0.7200	0.0250
553	0.0270	0.0303	0.7158	0.0250
554	0.0271	0.0302	0.7106	0.0250
555	0.0270	0.0302	0.7039	0.0250
556	0.0270	0.0300	0.6933	0.0250
557	0.0269	0.0300	0.6828	0.0250
558	0.0270	0.0299	0.6705	0.0250
559	0.0269	0.0298	0.6535	0.0250
560	0.0270	0.0297	0.6382	0.0251
561	0.0269	0.0297	0.6221	0.0250
562	0.0269	0.0295	0.5998	0.0249
563	0.0268	0.0294	0.5814	0.0249
564	0.0269	0.0294	0.5624	0.0250
565	0.0269	0.0293	0.5387	0.0249
566	0.0269	0.0292	0.5197	0.0249
567	0.0269	0.0290	0.5001	0.0250
568	0.0269	0.0291	0.4756	0.0249
569	0.0267	0.0290	0.4553	0.0249
570	0.0269	0.0290	0.4350	0.0249
571	0.0268	0.0290	0.4149	0.0250
572	0.0268	0.0288	0.3913	0.0249
573	0.0269	0.0288	0.3733	0.0249
574	0.0269	0.0287	0.3560	0.0249
575	0.0269	0.0288	0.3349	0.0251
576	0.0268	0.0288	0.3186	0.0251
577	0.0268	0.0287	0.3024	0.0250

[a] The measurement uncertainties (u) are: $u(A) = 10^{4}$ and $u(pH) = 0\,001$.

with growing tie-line length (TLL) for all the studied biomolecules, even for vitamin C, which presented decreasing partition coefficients with increasing TLL, as previously seen in Table 4. Similarly to what was done for the partition coefficients in Fig. 8, the extraction efficiencies (E) were plotted in function of the tie-line lengths (TLL), as Fig. 10 shows.

As seen in Fig. 10, the longest tie-lines provided the highest extraction efficiencies (E) due to more distinct compositions of the phases, which promote differences in important solvent properties for solute migration, such as polarity, density and dielectric constant. Vitamins B9 and B12 had larger extraction efficiencies than vitamin B2, which was

Table A11
Measured UV–vis absorbance from 200 to 600 nm at pH ≃ 7.5 for aqueous solutions of epicatechin (E) and vitamins B9, B12 and C at (15.4, 42.4, 31.2 and 7.60) · 10^{-5} g·mL ^{1}, respectively [a] - continuation.

Wavelength / nm	E	B9	B12	C
578	0.0269	0.0286	0.2827	0.0251
579	0.0269	0.0286	0.2671	0.0251
580	0.0269	0.0285	0.2519	0.0251
581	0.0269	0.0283	0.2340	0.0251
582	0.0269	0.0283	0.2204	0.0251
583	0.0268	0.0282	0.2077	0.0252
584	0.0268	0.0283	0.1928	0.0251
585	0.0268	0.0282	0.1815	0.0252
586	0.0267	0.0281	0.1709	0.0251
587	0.0268	0.0282	0.1583	0.0251
588	0.0269	0.0281	0.1495	0.0250
589	0.0269	0.0281	0.1411	0.0253
590	0.0269	0.0280	0.1315	0.0251
591	0.0268	0.0280	0.1240	0.0252
592	0.0269	0.0279	0.1172	0.0252
593	0.0270	0.0278	0.1088	0.0252
594	0.0269	0.0279	0.1029	0.0252
595	0.0269	0.0279	0.0961	0.0252
596	0.0269	0.0279	0.0915	0.0252
597	0.0269	0.0280	0.0872	0.0252
598	0.0270	0.0279	0.0822	0.0254
599	0.0271	0.0280	0.0784	0.0254
600	0.0271	0.0281	0.0746	0.0255

[a]The measurement uncertainties (u) are: $u(\mathrm{A}) = 10^{4}$ and $u(\mathrm{pH}) = 0\,001$.

the scope of a previous work of the research group [7]. Furthermore, the good linear relations obtained between the tie-line lengths and the experimental extraction efficiencies (Fig. 10) and between the tie-line lengths and the partition coefficients (Fig. 8) may allow to empirically predict their values for other tie–line compositions of the same ABS.

In Fig. 11, the extraction efficiencies of epicatechin and vitamin B12 were compared with the ones obtained with the ABS {ethyl lactate (1) + tripotassium citrate (K_3Citrate) or disodium tartrate (Na_2Tartrate) (2) + water (3)} at 298.15 K and 0.1 MPa in a previous work of the research group [30]. The ABS based on citrate salts provided higher extraction efficiencies and the separation of epicatechin was better in the ABS based on K_3Citrate, while the ABS composed of K_3Citrate and Na_3Citrate obtained very similar results for vitamin B12.

4. Conclusions

Antioxidant-rich dietary supplements could become especially important to tackle malnutrition in underdeveloped countries, since deficiencies in metals and antioxidants are widespread in these nations, promoting the appearance of diseases such as diabetes, anaemia and cancer. The most common feedstocks for natural antioxidants, *i.e.*, chemical species which delay the oxidative damage by reactive oxidative species, are fruits and vegetables, but the usage of food as raw material raises moral issues and contributes to several environmental and social problems, such as larger farming areas, water scarceness and even global hunger. Therefore, the use of antioxidant-rich biowaste is a more feasible feedstock for the production of food supplements, contributing simultaneously to a more circular economy and to more sustainable therapeutics.

In this work, the partition of epicatechin and of vitamins B9, B12 and C was carried out in the green Aqueous Biphasic System (ABS) {ethyl lactate (1) + trisodium citrate (2) + water (3)} at 298.15 K and atmospheric pressure for future valorisation of common biowastes, such as fruit pomaces (*e.g.,* from grapes) and vegetable peals pomaces (*e.g.,* from potatoes). The largest partition coefficients (K) and extraction efficiencies (E) were obtained for vitamin B12 ($K = 55\,44$, $E = 98\,5$ %) and epicatechin ($K = 5\,44$, $E = 84\,8$ %) for the longest tie-line (TLL = 69 68 %), while, in the same conditions, vitamin B9 ($K = 1\,48$, $E = 61\,6$ %) and vitamin C ($K = 0\,41$, $E = 32\,2$ %) presented smaller affinity for the ethyl lactate-rich phase. These results were obtained with low biomolecule mass losses in quantification, validating UV–vis spectroscopy as analytical method, and after a careful study of the biomolecule stages present for each pH value, which will be essential for future predictions of partition using thermodynamic modelling or molecular simulation.

CRediT authorship contribution statement

Pedro Velho: Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Leonor R. Barroca:** Investigation, Writing – review & editing. **Eugenia A. Macedo:** Conceptualization, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A

In the Appendices, the UV–vis absorbance spectra of all studied biomolecules and the variation of the respective mean electrical charges (q) with pH can be found (see Figs. A1-A4 and Tables A1-A11).

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