Algal Research 89 (2025) 104044

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Monitoring the presence of pigments in aqueous extracts using silica materials functionalized with ionic liquids

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A R T I C L E I N F O

Keywords:
Silica-based material functionalized with ionic liquid
Monitoring
Pigments
Algae

A B S T R A C T

Macroalgae are photosynthetic microorganisms living in saline environments. Included in the natural compounds produced by macroalgae are natural pigments and colorants such as chlorophylls, carotenoids and phycobiliproteins. These may be harvested directly in nature or cultivated in aquaculture systems. Indeed, a major challenge for aquaculture companies is the lack of standardized, accessible techniques that would allow for the qualitatively assessment of the presence of these pigments in algae biomass and derived extracts. Typically, identifying and measuring these pigments requires complex procedures that may be difficult to implement in smaller aquaculture companies in need of a quick and straightforward solution. This study addresses this gap by developing a silica-based material functionalized with ionic liquid for detecting the presence of phycobiliproteins and other pigments in aqueous extracts.

1. Introduction

Macroalgae are photosynthetic microorganisms living in saline environments, categorized by their coloration in three main species: red, brown and green macroalgae. Macroalgae are rich sources of various bioactive compounds, including pigments, carbohydrates, proteins, and lipids, with their composition varying widely among species and influenced significantly by the environmental conditions to which they are exposed during growth. The primary pigments found in macroalgae include chlorophylls, carotenoids, and phycobiliproteins [1], each imparting distinct colors and serving as valuable resources for industries such as food, cosmetics, and pharmaceuticals. To maximize the yield of the pigments produced, several studies have focused on optimizing cultivation conditions [2], such as light intensity, wavelength, temperature, nutrient availability, and salinity. Both academia and industry have invested significant efforts during the last few decades on the exploitation of value-added bioproducts that can be sourced from algae, and which can allow the development of a biorefinery focusing on a blue economy.

Phycobiliproteins are photosynthetic light-harvesting proteins found in cyanobacteria, red algae, and cryptomonads. These water-soluble proteins are bound covalently through cysteine amino acids to chromophores known as phycobilins, which are open-chain tetrapyrroles [1,3]. Phycobiliproteins are organized into supramolecular structures called phycobilisomes, located within the cell stroma [4]. All phycobiliproteins share a fundamental monomeric structure composed of α and β subunits. Based on structural characteristics and light absorption properties, phycobiliproteins are categorized into four main types: phycoerythrin, phycocyanin, phycoerythrocyanin, and allophycocyanin [5]. R-phycoerythrin is found in the red algae and cyanobacteria and it is generally composed of $(\alpha\beta)_6\gamma$ complexes (α, 18–20 kDa; β, 19.5–21 kDa; and γ, 30 kDa) with a total molecular weight around 240 kDa [6]. The increment of the γ subunit in phycoerythrin in comparison with other phycobiliproteins confers it an additional stability, since this subunit is located in the center of the molecule linking the $(\alpha\beta)_3$ trimers [7]. R-phycoerythrin (the one derived from red macroalgae) is commonly used as a natural coloring agent [8], fluorescent label probe [9] and as an ingredient in pharmaceutical formulations [10]. Many studies show the various biological activities of R-phycoerythrin, namely its antioxidant and anti-cancer properties [11–13].

Contrarily to what happens with phycobiliproteins, colorants with high solubility in water, chlorophylls and carotenoids are not, although they all share the high interest of industry for their application. In the field of aquaculture, the increasing economic value of natural colorants like phycobiliproteins has heightened the need for cultivation techniques optimized to enhance the pigment production in red macroalgae [3,5]. A major challenge for aquaculture companies is the lack of standardized, accessible techniques that would allow for the qualitative

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https://doi.org/10.1016/j.algal.2025.104044
Received 13 December 2024; Received in revised form 12 March 2025; Accepted 16 April 2025
Available online 28 April 2025

assessment of these pigments' presence in algae biomass and derived extracts. Typically, identifying and measuring these pigments requires complex procedures (and sometimes expensive equipment) involving sample disruption, extraction, centrifugation, UV–Vis spectroscopy and/or chromatographic steps that may be difficult to implement in smaller aquaculture companies in need of a quick and straightforward solution.

This study addresses this gap by developing a lab-scale approach using a silica-based material functionalized with ionic liquid for detecting phycobiliproteins in aqueous extracts. Silica-based materials functionalized with ionic liquids have demonstrated versatility in applications ranging from adsorption and separation to extraction, making them promising candidates for pigment detection in aquaculture contexts. These materials' adaptability for adsorption processes in batch, semi-continuous, and continuous processing has provided flexibility for various applications including proteins, metals and anti-inflammatory drugs from aqueous based matrices and separation of different compounds in complex extracts [14–17].

The adsorption phenomenon used in this study leverages mass transfer principles, with molecules or atoms binding to a solid surface through either physical (e.g., Van der Waals, hydrogen bonding, ion exchange) or chemical interactions, providing a viable pathway for detection. The use of solid materials in solid-phase extraction is particularly promising due to its cost-effectiveness, simplicity, and efficiency [18,19], making it an ideal approach for the development of a simple qualitative pigment detection. Additionally, ionic liquids have gained attention for their tunable chemical properties and adaptability across diverse applications, including those combined with silica and other supports such as polymers, magnetic carboxymethyl cellulose, and single-walled carbon nanotubes [20]. Building on prior successes with ionic liquid-functionalized silica, this study aims to develop an efficient, straightforward material for detecting phycobiliproteins in aqueous media. This work began with the preparation of a simple phycobiliprotein extract from *Gracilaria gracilis* using conventional methods. Following extraction, different silica-based materials functionalized with four distinct ionic liquids were tested for their ability to adsorb phycobiliproteins from the extract in both batch and semi-continuous modes. Finally, elution methods were evaluated to study desorption efficiency, examining the potential for multiple cycles of use. Based on the results, this standardized process shows promise for monitoring phycobiliproteins in aqueous extracts, as well as identifying other pigments, more hydrophobic, that may be present. This approach could be particularly valuable for aquaculture companies interested in tracking the accumulation of key pigments, thus supporting more efficient and environmentally sustainable production processes.

2. Materials and methods

2.1. Materials

Silica gel (60 Å) with a particle size of 0.2–0.5 mm was purchased at Merck. Hydrochloric acid (HCl, 37 wt% of purity), cholinium chloride (98 % of purity) and triethylamine (≥99.5 %) were bought to Sigma-Aldrich. The solvents used to prepare the IL-functionalized silica materials were toluene (99.98 % purity), ethanol (99.99 % of purity) and methanol (99.99 % of purity) all from Fisher Scientific. The (3-chloropropyl)trimethoxysilane (98 % of purity), *N*-methylimidazole (99 % of purity), and tributylamine (99 % of purity) provided by Acros Organics. Trioctylamine (>98 % of purity) was acquired from Fluka.

2.2. Gracilaria gracilis extract

Fresh *Gracilaria gracilis* (*G. gracilis*) was provided by ALGAplus – Produção e Comercialização de Algas e seus Derivados, Lda, located in Ílhavo, Portugal. After collecting the macroalga, the biomass was cleaned, washed and stored in a freezer at −20 °C. Three batches were used for this study, produced in April 2021, June and November 2022. Twenty grams of fresh *G. gracilis* were macerated using a mortar and pestle while frozen with liquid nitrogen, homogenized in 40 mL of distilled water at room temperature and placed in an incubator shaker (IKA KS 4000 ic control) for 20 min, at 250 rpm and room temperature. After, the solution was filtered and centrifuged in a Thermo Scientific Heraeus Megafuge 16 R Centrifuge at 3500 rpm for 30 min, at 4 °C, thus resulting in an extract rich in phycobiliproteins, and mainly in R-phycoerythrin, since this is the one most abundantly produced by this alga.

2.3. Silica-based materials functionalized with ionic liquids

The synthesis of the four silica-based materials functionalized with the ILs was already reported in literature [14,16]. Briefly, four different ionic liquids were used to functionalize the silica: the 1-methyl-3-propylimidazolium chloride ($[Si][C_3C_1Im]Cl$), triethylpropylammonium chloride ($[Si][N_{3222}]Cl$), tributylpropylammonium chloride ($[Si][N_{3444}]Cl$), and trioctylpropylammonium chloride ($[Si][N_{3888}]Cl$). The synthesis of these materials involved two main steps: the (i) activation of commercial silica gel 60 (0.2–0.5 mm) using hydrochloric acid (37 wt%) for 24 h to increase the silanol group content on the silica surface, and the (ii) silica functionalization. For the functionalization of silica with ionic liquids, a first reaction was applied using a silane-coupling agent that also provides the anion (3-chloropropyltrimethoxysilane, $C_6H_{15}ClO_3Si$). For this step, 5 g of activated silica were suspended in 60 mL of toluene, with 5 mL of $C_6H_{15}ClO_3Si$ added, and the suspension was kept under reflux with magnetic stirring for 24 h. The resulting solid was filtered and washed in sequence with 100 mL of toluene, 200 mL of ethanol (1:1 v/v), 500 mL of distilled water, and 100 mL of methanol, then dried for 24 h at 60 °C, yielding silica with a chloropropyl spacer arm ($[Si][C_3]Cl$). After, a second reaction was done, with 5 g of $[Si][C_3]Cl$ being mixed with 60 mL of toluene and 5 mL of the cation source, e.g., *N*,*N*-dimethylbutylamine. The suspension was kept under reflux with magnetic stirring for 24 h. The material was then filtered and washed in sequence with 100 mL of toluene, 350 mL of methanol, 300 mL of distilled water, and 150 mL of methanol, and subsequently dried for 24 h at 60 °C. Elemental analysis for the determination of carbon, hydrogen, and nitrogen contents (weight percentage) was performed using a Truspec Micro CHNS 630-200- 200 elemental analyzer and following the protocols described in literature.

2.4. Pigments adsorption in the silica functionalized materials

The adsorption of phycobiliproteins in the silica functionalized materials was conducted using 50 mg of functionalized silica and 5 mL of *G. gracilis* extract at room temperature. The mixture was placed in an incubator shaker (IKA KS 4000 ic control) for 1 h at 150 rpm and room temperature. Following incubation, the solution was centrifuged at 10,000 rpm for 15 min using a Thermo Scientific Heraeus Megafuge 16 R centrifuge. The study evaluated the four silica-based materials, namely $[Si][C_3C_1Im]Cl$, $[Si][N_{3222}]Cl$, $[Si][N_{3444}]Cl$, and $[Si][N_{3888}]Cl$. R-Phycoerythrin content was quantified by measuring the absorbance of the samples within the UV–visible spectrum (200–700 nm) using a Synergy HT microplate reader (BioTek) and using a calibration curve previously done. For adsorption kinetics, the same procedure was followed, with measurements taken every 5 min for a total contact time of 70 min. The equilibrium concentration of the adsorbate on the solid phase was calculated using Eq. (1) [22]:

$$q_e = \frac{(C_o - C_e) \times V}{w} \tag{1}$$

where w is the mass of the silica functionalized with ionic liquid (g), V is the volume of the *G. gracilis* extract (mL), C_0 and C_e ($mg.mL^{-1}$) are the equilibrium concentrations of the solution before and after the adsorption process, respectively.

2.4.1. Optimization of the R-phycoerythrin adsorption

Central composite rotatable design (CCRD) was applied to optimize the R-phycoerythrin adsorption, and three independent variables were studied: added mass of silica-based materials (X_1), contact time (X_2) and volume of the macroalga extract (X_3). It's important to note that the concentration of R-phycoerythrin in the extract was not optimized, as the extract obtained from the alga sample and used was already close to its maximum concentration. A 2^3 factorial design was employed, incorporating six central and axial points, resulting in a total of 20 experimental runs. This analysis was performed for the silica/IL-based material with high capacity to absorb R-Phycoerythrin. The results were analyzed using the response surface methodology, residual analysis, and a 95 % confidence level, with pure error serving as the standard. Adsorption efficiency (AE - %) was used as the dependent variable (Eq. (2)). Table 1 presents the independent variables, their coded levels, and design coordinates. Data analysis was conducted with Statistica® 7 software, and optimal adsorption conditions were determined and verified in triplicate using the relative deviation (%).

$$AE\ (\%) = \frac{C_O - C_1}{C_O} \times 100 \quad (2)$$

C_o is the initial concentration of R-phycoerythrin in the extract and C_1 is the final concentration of R-phycoerythrin in the extract.

2.5. (Semi-continuous) adsorption of phycobiliproteins in the silica/IL-based material

The (semi-continuous) adsorption process was conducted using a column equipped with a peristaltic pump (Bio-Rad Econo-Pac). The setup contained 2 g of silica/IL-based material positioned between two frits within a 20 mL empty polypropylene cartridge. While the proportions from the batch process were initially attempted, additional silica/IL-based material was necessary to achieve effective performance. The process began by passing 25 mL of extract through the column, containing an initial R-phycoerythrin concentration of 0.0179 $mg.mL^{-1}$, followed by 25 mL of a cholinium chloride (1 M) aqueous solution to facilitate R-phycoerythrin desorption (proved to be efficient eluting this phycobiliprotein [23]) and test the possibility of reusing the silica-based material in new cycles. A flow rate of 52 $\mu L.s^{-1}$ was maintained throughout. The R-phycoerythrin content, for both adsorption and desorption phases, was quantified by measuring the UV–visible absorption between 200 and 700 nm using a UV–Vis microplate reader (Synergy HT microplate reader-Biotek). The R-phycoerythrin mass used in the calculation of adsorption and desorption was calculated, respectively, by determining the amount of R-phycoerythrin retained in the resin and eluted with 1 M of cholinium chloride (see Fig. S1 in Supporting information). Adsorption and desorption efficiencies were calculated through Eqs. (2) and (3), respectively. The semi-continuous adsorption experimental apparatus is provided in Fig. S2 in Supporting information. In the end, a new experiment was conducted for a larger scale, in which the experimental procedure here described was followed, but using 5 g of the silica-based material and 45 mL of extract.

$$DE\ (\%) = \frac{m_{des}}{m_{ads}} \times 100 \quad (3)$$

m_{des} is the mass of R-phycoerythrin in the eluted solution, m_{ads} is the total mass of R-phycoerythrin adsorbed onto the silica-based materials.

Table 1
Factors and levels used for the CCRD (2^3).

Variables	Code	Axial point	Factorial point	Central point	Factorial point	Axial point
		Coded variable level				
		−1.68	−1	0	1	1.68
Mass of silica (mg)	X1	43.20	50.00	60.00	70.00	76.80
Contact time (min)	X2	13.03	19.9	30.0	40.1	47.0
Volume of extract (mL)	X3	5.01	7.8	12.0	16.2	19.0

2.6. Statistical analysis

The results were expressed as the means ± standard errors of the mean. Statistically significant differences were determined considering an α of 95 % (*p-value* < 0.05).

3. Results

The main objective of this study was to develop a straightforward method for monitoring the presence of phycobiliproteins in aqueous extracts. To achieve this, the work was structured in two main parts. In the first part, we tested the adsorption capacity of silica-based materials functionalized with four different ionic liquids (elemental analysis provided in Table S1 from Supporting information) to adsorb the phycobiliproteins. After identifying the most efficient material for phycobiliprotein adsorption in a batch process, its performance in a semi-continuous adsorption process was tested to evaluate whether the simple passage of the extract through the material could effectively monitor the phycobiliproteins' presence. Following the successful demonstration of phycobiliprotein monitoring using the most efficient silica/IL-based material, the pigment's elution was tested to explore the potential for the repeated use of the material.

3.1. Pigments adsorption in the silica/IL-based materials

The initial step of this work involved assessing the ability of silica/IL-based materials, each functionalized with one of four different ionic liquids—$[Si][C_3C_1Im]Cl$, $[Si][N_{3222}]Cl$, $[Si][N_{3444}]Cl$, and $[Si][N_{3888}]Cl$—to adsorb phycobiliproteins from an aqueous extract. An extract rich in phycobiliproteins (with R-phycoerythrin being the most abundantly present) was prepared from *G. gracilis* produced in April 2021. The extract obtained had an initial concentration of 0.0307 $mg.mL^{-1}$ in R-phycoerythrin. To note that all the experiments in which the concentration of phycobiliproteins is mentioned refer to the concentration of R-phycoerythrin since this is the most abundantly produced by this alga, unless something is specifically mentioned. Each silica-based material was exposed to the extract for 60 min, followed by centrifugation and analysis of the resulting material (Fig. 1A). As seen in Fig. 1A, all samples show the silica settled at the bottom after centrifugation, but only in Fig. 1A, the one representing the $[Si][C_3C_1Im]Cl$-based material, the extract appears completely colorless. This indicates that, of the four materials tested, only the silica functionalized with the imidazolium ionic liquid $[Si][C_3C_1Im]Cl$ achieved complete pigment adsorption. These findings are also corroborated by the results obtained for the UV–Vis spectroscopy (Fig. 1B), since only for this system the extract does not present absorbance for any wavelength tested.

Moreover, and by observing the results described in Table 2, where the values of q_e and AE are described, it is possible to see that the AE of this extract is actually around 94 %, showing its highest performance when compared with the others (from 16.5 % to 18.7 %) and it reaches the equilibrium at 0.0029 $mg.g^{-1}$. The better performance of $[Si][C_3C_1Im]Cl$ may be explained by the higher compatibility of R-phycoerythrin, a hydrophilic pigment, with the most hydrophilic ionic liquid used to functionalize the silica, or even by the hydrophilic interactions taking place, namely potential $\pi - \pi$ interactions, electrostatic interactions, Van der Waals forces, and hydrogen bonding that may be

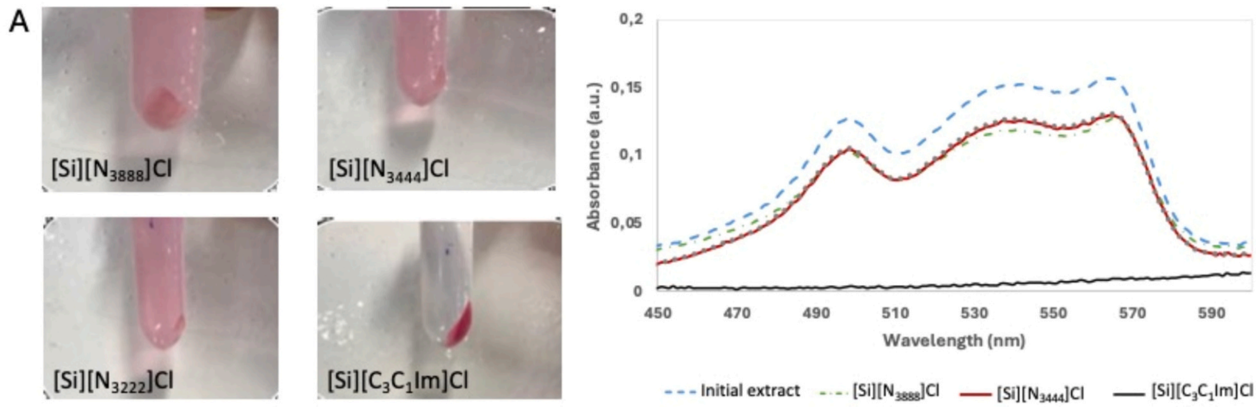

Fig. 1. (A) Picture of the silica-based materials functionalized with the different ionic liquids after being in contact with the phycobiliproteins extract for 60 min; (B) UV–Vis spectra obtained for the extract of phycobiliproteins after being in contact with the different silica-based materials.

Table 2
Data for concentration of the extract rich in R-phycoerythrin, q_e, and AE for the four different silica-based materials after being in contact with the R-phycoerythrin extract, for 60 min.

SILs	R-phycoerythrin concentration ($mg.mg^{-1}$)	q_e ($mg.g^{-1}$)	AE (%)
$[Si][N_{3222}]Cl$	0.0249	0.0006	18.7
$[Si][N_{3444}]Cl$	0.0254	0.0005	17.1
$[Si][N_{3888}]Cl$	0.0256	0.0005	16.5
$[Si][C_3C_1Im]Cl$	0.0020	0.0029	93.6

Table 3
Kinetic parameters for the pseudo-first order and pseudo-second order models associated to the adsorption of R-phycoerythrin.

Pseudo-first order model	q_e ($mg.g^{-1}$)	0.02165
	k_1 (min^{-1})	0.3760
	R^2	0.9861
Pseudo-second order model	q_e ($mg.g^{-1}$)	0.02252
	k_2(min^{-1})	40.12
	R^2	0.9953

established between both the R-phycoerythrin and the $[Si][C_3C_1Im]Cl$, as also reported in studies on the favorable interactions between other proteins and imidazolium-based ILs [24].

After the selection of the most efficient silica/IL-based material for the adsorption of phycobiliproteins - the one functionalized with $[Si][C_3C_1Im]Cl$ - the kinetic of adsorption was evaluated, through two different models (Fig. 2), the pseudo-first-order Lagergren model [25] and the pseudo-second-order Ho model [26]. The systems were prepared by using 50 mg of $[Si][C_3C_1Im]Cl$, placed in contact with the phycobiliproteins extract (concentration of R-phycoerythrin of 0.0307 $mg.mL^{-1}$) for 60 min. A comparison between the experimental data and the model fits is presented in Fig. 2, and the corresponding kinetic parameters are summarized in Table 3.

As observed by the adsorption kinetic curve, the equilibrium was reached in 10 min, being kept up at least 70 min. From the results of the correlation coefficients (R^2) obtained for each model in Table 2, it is concluded that the pseudo-second order model is the one best describing

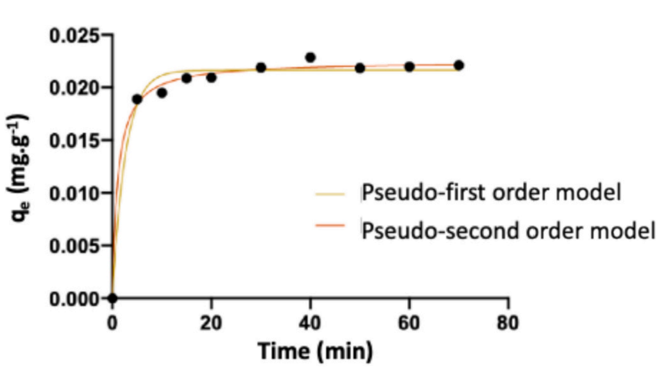

Fig. 2. Kinetic curve/sorption isotherms and respective kinetic models adjusted for the adsorption of R-phycoerythrin at room temperature. The black dots represent the experimental values determined in this work.

the adsorption of R-phycoerythrin by $[Si][C_3C_1Im]Cl$. These results indicate that the adsorption mechanism is determined by strong chemical interactions at the liquid-solid interface of the adsorbent [27]. Considering the absence of good adsorption results for other silica-based materials, no kinetic model could be applied. Nevertheless, the same second-order kinetic model was also observed for the adsorption of other compounds, although not proteins, specifically platinum and palladium from aqueous media.

3.1.1. *Optimization of the R-phycoerythrin extraction*

After selecting the best material for the adsorption of R-phycoerythrin, $[Si][C_3C_1Im]Cl$, the adsorption mechanism was further optimized. New extracts rich in R-phycoerythrin were prepared but in this case, obtained from a different batch of *G. gracilis*. For these experiments, the batch used was the one collected in June 2022, which allowed to obtain an initial extract with 0.0327 $mg.mL^{-1}$ of R-phycoerythrin. The conditions under optimization were the mass (mg) of $[Si][C_3C_1Im]Cl$ (X_1), the contact time (min) between the material and the extract rich in R-phycoerythrin (X_2), and the volume of extract (mL) (X_3) based on a central composite rotatable design (CCRD-2^3). Twenty experimental runs, including five central and axial points at levels −1.68 and +1.68, were conducted to evaluate the adsorption efficiency (%), which served as the dependent variable for the predictive model. Table S2 in Supporting information presents the AE of R-phycoerythrin in the $[Si][C_3C_1Im]Cl$ along with the respective extraction conditions tested. The fitted model described in Eq. (4), obtained using the analysis of variance (ANOVA) to indicate the statistical significance of the variables and their interactions, has demonstrated good predictability at a confidence level of 95 % with $R^2 = 0.81905$, and F-calculated > F-tabulated.

$$AE\,(\%) = 75.65 + 0.15(X_1) - 0.59(X_2) - 6.17(X_3) + 0.12(X_3)^2 + 0.07(X_3 \times X_3) \tag{4}$$

The AE ranged from 24.3 % in assay 6 to 49.3 % in assay 9 (Table S2). Response surfaces plotted in Fig. 3 show that the optimal extraction conditions—76.8 mg of $[Si][C_3C_1Im]Cl$, 5 mL of extract, and 47 min of contact time yielded the highest adsorption efficiency of at circa 49.34

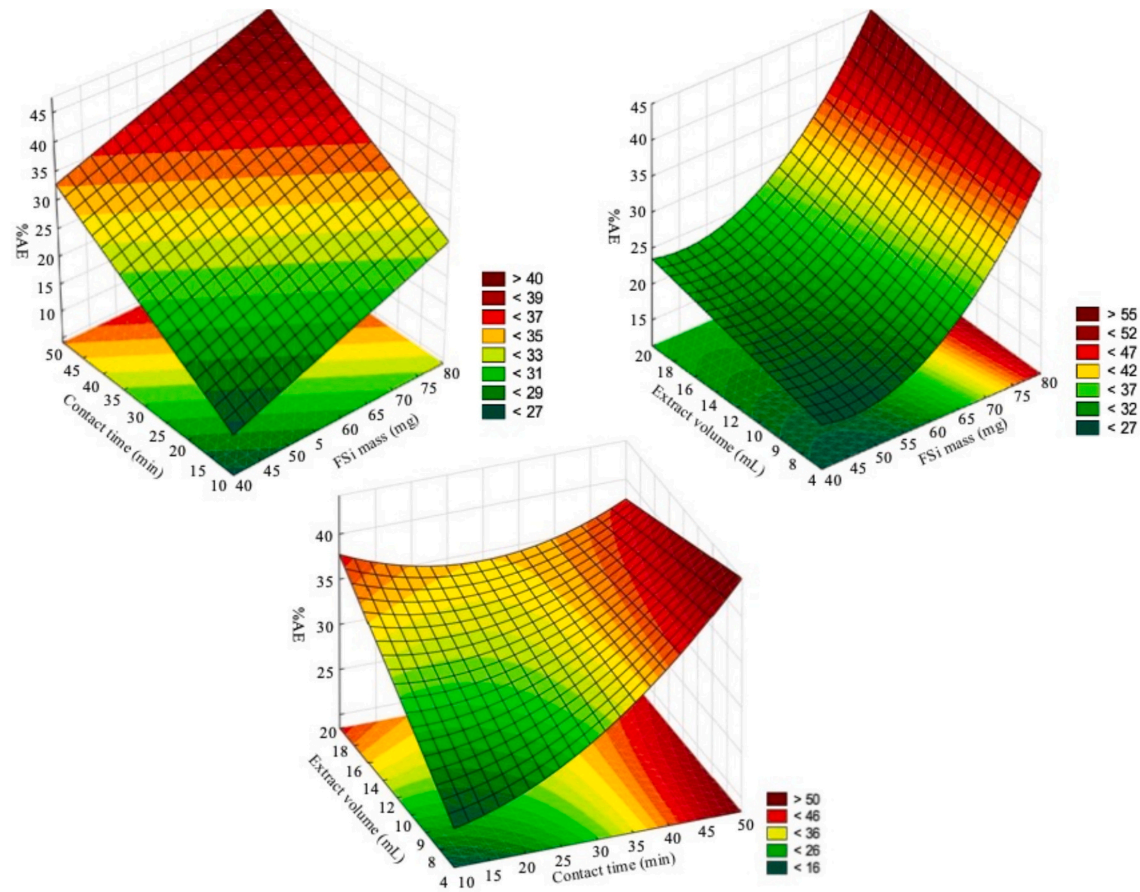

Fig. 3. Response surface plots obtained for the CCRD (2^3) of the adsorption efficiency of R-phycoerythrin (%). The independent variables considered were the mass of [Si][C_3C_1Im]Cl (mg), the contact time (min) and the volume of extract (mL).

%. The results suggest that using the smallest extract volume tested yields the highest efficiency, making it the preferred choice. This approach not only reduces the amount of alga needed but also minimizes the volume of water required in the preparation of the extract.

The accuracy and precision of the model were validated through an experimental test conducted under the optimal operational conditions, yielding a low deviation (6.47 %) from the predicted results (Fig. S3 in Supporting information). The Pareto chart (Fig. S4 in Supporting information) indicates that the volume of extract is the most significant variable affecting the adsorption efficiency of R-phycoerythrin in [Si][C_3C_1Im]Cl, though other variables also play an important role in the optimization process. The alignment between predicted and observed values (Fig. S3 in the Supporting information) further supports the model's reliability.

In the tests previously conducted in this study, we observed a higher AE compared to the result obtained here in the optimization. This variation may be attributed to differences in the biochemical composition of the *G. gracilis* macroalgae used, as the first sample was harvested in April 2021 and the second in June 2022, differences previously reported by some groups working in the field for different algae species [29,30]. In this context, although the concentration of R-phycoerythrin in the extract remains nearly the same despite being prepared from two different batches, the other co-extracted compounds, namely non-fluorescent proteins, may have negatively influenced the adsorption mechanism during the optimization process. Although non-fluorescent proteins were not characterized in this work, their presence was already briefly reported by us [31] and others [32]. Nonetheless, this optimization allowed us to determine the most efficient conditions for performing the adsorption.

3.2. (Semi-continuous) adsorption of R-phycoerythrin using the silica functionalized with [Si][C_3C_1Im]Cl

The adsorption process for R-phycoerythrin was conducted in semi-continuous mode using a solid-phase extraction column containing [Si][C_3C_1Im]Cl and with the extract rich in R-phycoerythrin (0.0270 mg.mL^{-1}) entering in the column at a flow rate of 52 μL.s^{-1}. For the development of these tests, the extract was prepared from a third batch, *G. gracilis* collected in November 2022. This setup was employed to adsorb and desorb R-phycoerythrin from the [Si][C_3C_1Im]Cl, as well as to evaluate the reusability of the material. Five cycles were performed, and the efficiency of both the adsorption (Fig. 4A) and desorption processes (Fig. 4B) was assessed.

As illustrated in Fig. 4A, the first adsorption cycle achieved nearly 100 % of AE, indicating effective adsorption. In subsequent cycles, up to the fifth, a slight increase in AE was observed, likely due to residual R-phycoerythrin retained within the extraction column from the second cycle onward. Desorption efficiency followed a similar trend (Fig. 4B): the first cycle exhibited 100 % efficiency, with a subsequent 20 % increase in later cycles. The solid-phase extraction method here proposed using [Si][C_3C_1Im]Cl as the adsorbent, proved to be highly effective for the R-phycoerythrin extraction, as all content in R-phycoerythrin was successfully adsorbed. Moreover, since efficiency was maintained across cycles, we can conclude that the adsorbent material, [Si][C_3C_1Im]Cl, could be reused lowering the costs of the method. Despite a 20 % increase in the amount of R-phycoerythrin observed in the second cycle, possibly due to some accumulation of this protein in the [Si][C_3C_1Im]Cl, this value does not continue to rise through to the fifth cycle, indicating that no further accumulation occurs with each subsequent cycle. To further validate the efficiency of [Si][C_3C_1Im]Cl, the same procedure

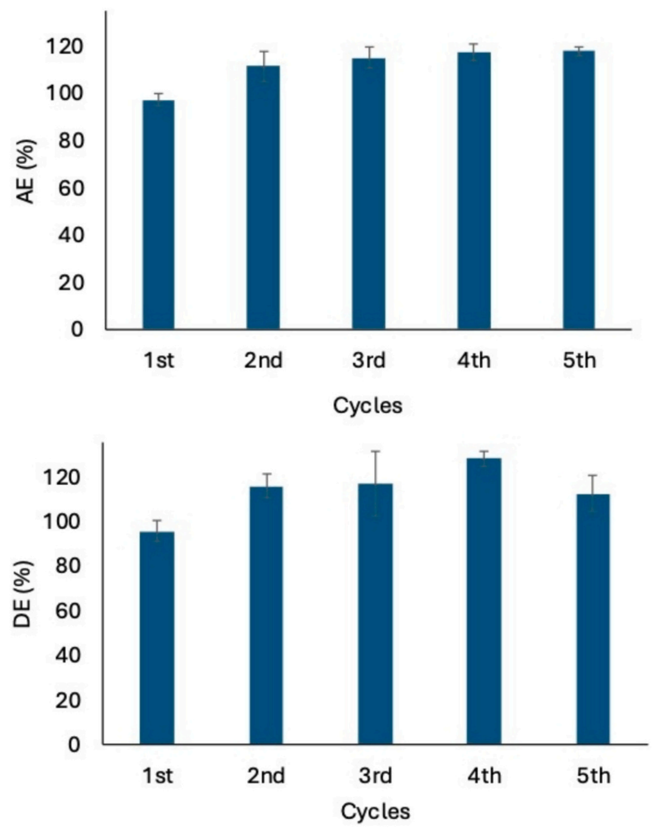

Fig. 4. Efficiency of adsorption and desorption (respectively, AE and DE) of R-phycoerythrin in [Si][C_3C_1Im]Cl for five consecutive cycles.

was conducted using a non-functionalized silica as adsorbent material. Fig. 5 displays the 25 mL of extract after it passed in the column packed with non-functionalized and functionalized silica. In Fig. 5a, there is no observable color change with [Si][C_3C_1Im]Cl, whereas Fig. 5b highlights the pink color of R-phycoerythrin. For the non-functionalized silica, the AE was approximately 40 %, while [Si][C_3C_1Im]Cl achieved 100 %, consistent with previous observations.

3.2.1. (Semi-continuous) adsorption of R-phycoerythrin in larger scale

After optimizing the semi-continuous adsorption process and confirming the need of [Si][C_3C_1Im]Cl for the effective adsorption of the R-phycoerythrin, the same process was implemented in larger scale. In here, the extract rich in R-phycoerythrin (0.0307 mg.mL^{-1}) was prepared with algae collected in April 2021. The results are illustrated in Fig. 6. After 15 mL of extract had passed through the column, the expected pink hue of R-phycoerythrin appeared, reflecting the strong affinity of this adsorbent material for this fluorescent protein. Simultaneously, the blue color of R-phycocyanin also began to appear, indicating that the [Si][C_3C_1Im]Cl material can be used to detect not only the predominant pink fluorescent protein produced by *G. gracilis* but also the blue protein, though present in a more limited amount due to its naturally lower production by the alga. As the process proceeded, an additional 5 mL of extract was introduced, after which the residual amount of R-phycocyanin was eluted from the column, but at a certain point, other colors appeared. After the 45 mL of extract prepared passed through the column, three distinct colors were observed on the adsorbent material: a bright pink representing a purer form of R-phycoerythrin, a purple layer that may indicate a mixture of R-phycocyanin and R-phycoerythrin, which may also be accumulating some allophycocyanin (also produced by this alga), as previously demonstrated by our group [31] and a greenish color indicating also the presence of some amount of chlorophylls in the extract.

The use of adsorption processes has previously been applied to separate pigments from algal extracts. For instance, Vaz and co-authors [23] employed a commercial resin (AmberLite) to separate chlorophylls from carotenoids, both classes present in extracts obtained from the microalga *Isochrysis galbana*, using ionic liquids for elution. Similarly, [33] successfully adsorbed β-carotene and lutein with triacontyl silica (CLEAN-UP) cartridges during sample pretreatment for HPLC analysis, while preconcentrated red wine pigments on cartridges packed with an octadecylsilica sorbent [34]. In these studies, the primary goal was the pigment fractionation or pre-concentration. A simple apparatus packed with the [Si][C_3C_1Im]Cl material developed in this study, may be implemented in aquaculture companies as a way of rapidly test the presence of pigments in aqueous extracts. Nevertheless, and while measuring the UV–Vis spectrum of an extract may seem like a simpler approach, the innovation of our process lies in its ability to qualitatively determine the composition of any pigment-containing extract, regardless of the pigment's nature—like how Thin-Layer Chromatography is used for the qualitative identification of lipids in lipidic extracts. Furthermore, this method and material allows not only to monitor the

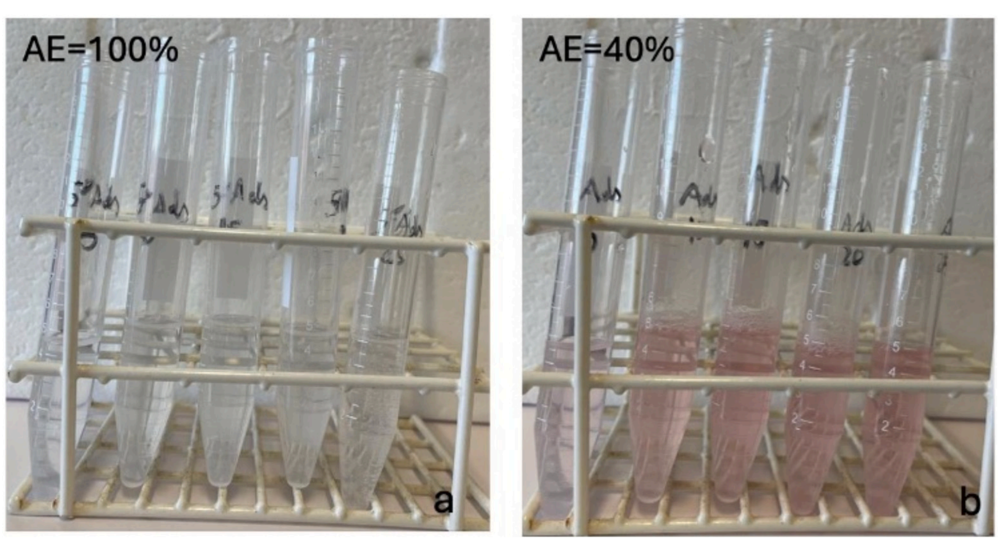

Fig. 5. Picture of the fractions collected from the extract rich in R-phycoerythrin after adsorption process using as the adsorbent material (a) [Si][C_3C_1Im]Cl and non-functionalized silica (b).

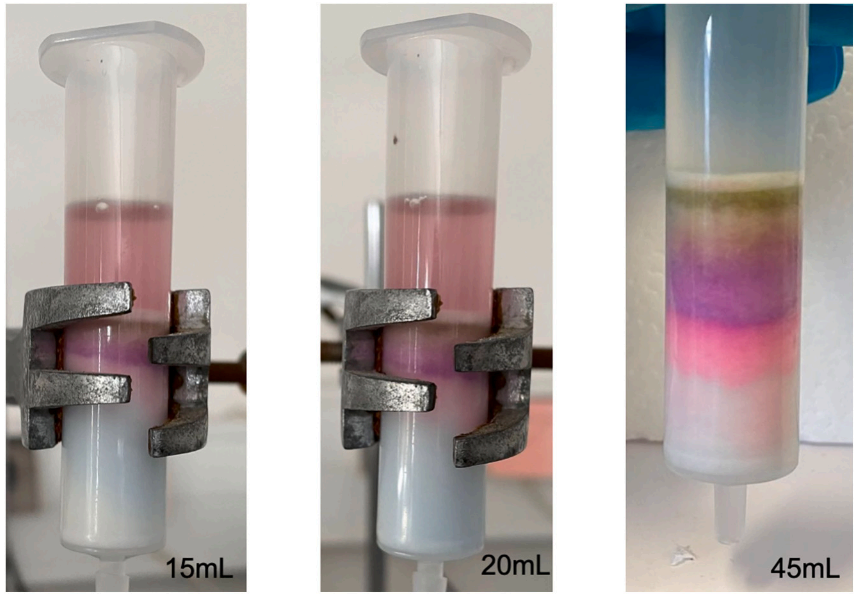

Fig. 6. Semi-continuous adsorption of R-phycoerythrin aqueous extract obtained from *G. gracilis* in larger scale.

primary pigment (in this work phycobiliproteins) but also the detection of contaminant pigments (in the present work chlorophylls) without the need for multiple calibration curves or pigment standards, since the goal is only to obtain a rapid monitoring of the extract composition, and thus to obtain a qualitative analysis. This capability is particularly valuable for companies that frequently need a quick qualitative assessment of their production process and extracts produced. In this sense, this proposed method/material provides a quick but qualitative approach for detecting different pigments (hydrophobic and hydrophilic) in a single aqueous extract.

4. Conclusions

In conclusion, this study addresses a significant challenge in aquaculture by developing an accessible and efficient method and material for detecting key pigments in algae aqueous extracts. This study aimed to apply an adsorbent material capable of identifying a range of pigments including phycobiliproteins, chlorophylls, and carotenoids, directly from aqueous extracts. The results indicate that this material can effectively monitor both hydrophilic pigments, such as R-phycocyanin and R-phycoerythrin, and hydrophobic pigments, like chlorophylls, without requiring the solvent change. This offers aquaculture companies a quick, straightforward, and solvent-free material for assessing pigment presence without requiring complex equipment or procedures. By providing this simple and cost-effective alternative for pigment monitoring, this work supports aquaculture companies' efforts to optimize quality control and streamline monitoring processes in algae cultivation.

CRediT authorship contribution statement

Gabriela Kovaleski: Writing – original draft, Methodology, Investigation, Formal analysis. **Márcia C. Neves:** Writing – review & editing, Methodology. **Sónia P.M. Ventura:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization.

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 or represent any conflict of interest.

Acknowledgments

This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020 (DOI:10.54499/UIDB/50011/2020), UIDP/50011/2020 (DOI:10.54499/UIDP/50011/2020) & LA/P/0006/2020 (DOI:10.54499/LA/P/0006/2020), financed by national funds through the FCT/MCTES (PIDDAC). GK thanks the financial support through her PhD grant: BD/REITORIA/9328/2020. M.C.N. acknowledges FCT for the research contract CEECIND/00383/2017/CP1459/CT0031 (DOI:10.54499/CEECIND/00383/2017/CP1459/CT0031) under the Scientific Employment Stimulus - Individual Calls.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2025.104044.

Data availability

Data will be made available on request.

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