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Title: Fluorescent responsive chlorophyllide-hydrogel for carbon dioxide detection

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Keywords: CO2, fluorescence, chlorophyllide b, sensor, raman, ss-NMR

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Abstract: The chlorophyllide b derivatized polyallylamine (PAAChl) was synthesized. The resulting fluorescent polymer presents a high emission quantum yield, very stable in time. The polymer formed a hydrogel when treated with carbon dioxide that was characterized by Raman spectroscopy and solid-state NMR (ss-NMR). The adsorption of CO2 takes place through the formation of carbamate. Absorbance and fluorescence based analytical studies were performed. The UV-vis spectra of PAAChl, after additions of CO2, show a growing absorption peak at 250 nm corresponding to carbamate formation. The emission of fluorescence at 664 nm as function of carbon dioxide concentrations shows a linear regression with a detection limit of 2 ppm and a quantification limit of 5 ppm. The capacity of a chemical sensor was described by sensibility, selectivity, stability, response time and regeneration were also analyzed.

***Response to Reviewers**

June 21th, 2016

Dear Editor of Sensors & Actuators: B. Chemical

Prof Ramaier Narayanaswamy,

We are sending the modified manuscript entitled: "Fluorescent responsive chlorophyllide-hydrogel for carbon dioxide detection". We are very grateful to the reviewers for their comments and suggestions and we hope with the modification we have done it meets the requirements of the Journal.

The changes appear in red in the revised manuscript attached as supplementary material.

Regards,

Prof. Irene Rezzano

Reviewers' comments:
(Response to reviewer is in bold italics letter)

1-The chlorophyllide mixture is not characterized, so it is no certitude to have a constant composition. Extracts of porphyrin derivatives from plants differ in composition and mixtures of 4-5 chlorophyll derivatives might be contained. This is a variable that put into question the whole research.

We thank the reviewer for pointing this out. We weren´t clear enough in the experimental section. Instead of the sucrose separation, we have done a silica gel 60 (0.063-0.2000mm) column with a gradient mobile phase, varying the proportions of dichloromethane and methanol (0.5%, 1.0%, 1.5%, 2.0% and 8%). Therefore, we have a 95-98% of chlorophyllide b in the solid extract.
We have modified the manuscript accordingly.

2-The H-NMR spectrum did not succeed to put into evidence the signals of these chlorophyll derivatives, that is a major issue, because, even when grafting a polymer only with 1-2% of porphyrin derivatives(as functionalities), 1H-NMR signals are still of high quality (read recent literature regarding polymer-porphyrin hybrid materials for CO2 detection.

We thank the reviewer for noticing this issue. However, we have calculated the chlorophyllide concentration and reported it in our previous work (Sensors and Actuators B 145 (2010) 250–253). In that paper we calculated that the amount of chlorine in the final polymer was 2.5 × 10^{-3} mg/mg, a 0.25%. Therefore, we couldn´t detect the chlorine bands in the NMR spectra.
Regarding ^{1}H-NMR spectra, it is necessary to point out that these kinds of simple experiments are not determined as routine in the solid state because the dipolar coupling among protons is higher than the commonly spinning speed obtained

by the commercial NMR probes (10-35 KHz). However, partially or well resolved ^{1}H spectra can be obtained at high spinning rate (>60 KHz) or with particularly high power decoupling techniques at moderate spinning rate. In general, proton spectra of solid polymer samples consist in wide lines and in some cases an overlapping of wide and sharp lines at static conditions.
Fortunately, for hydrogels there is an experiment called high-resolution magic angle spinning (HRMAS), where the ^{1}H-^{1}H dipolar interactions can be partially averaged, rendering liquid-like ^{1}H-NMR spectra similar to those observed for liquids. In this technique, the material is swelled with deuterated solvents, making it possible to expand the analysis of hydrogel compounds.
However, in this work it was difficult to access the segments where the chlorophyllide is bounded maybe as a consequence of a low swelling of that section or only to a sensitive problem. The idea of this HRMAS experiment was to demonstrate the gel behavior after the carbamate group formation due to the interaction between the PAAChl and CO_2. The chlorophyllide bounded to PAA polymer is demonstrated with the UV and raman spectra.

3-It is not clearly explained in the text if the emission spectra are from solution or from gels. But if the material transforms into gel as response to CO2 exposure, what kind of material is studied?

The polymer transforms into a gel as a response to the exposure to CO2. The emission spectra studied are from the solution containing the polymer. The gel was then analysed to confirm the formation of the carbamate and to characterize the product.

4- A reaction scheme is needed.

We added a Scheme for the polymer modification reaction following the reviewers´ advise.

5- References are old (only a few from the last 5 years) although there are tens of novel papers published in CO2 detection (on diverse materials polymer porphyrin ones, silica-porphyrin hybrids, bare porphyrins and metalloporphyrins alone).

We have added some references following the reviewers´ advice.

6- The fluorescence quantum yield was not determined, but it is stated in the highlights.

We thank the reviewer for noticing this point. We have corrected the highlights accordingly.

*Manuscript
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Fluorescent responsive chlorophyllide-hydrogel for carbon dioxide detection

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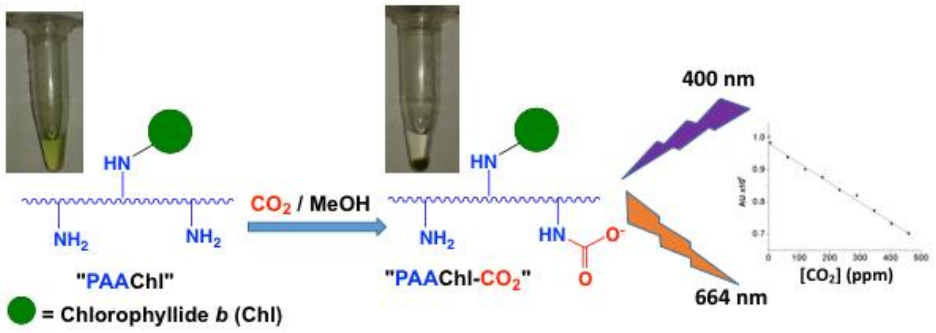
Graphical Abstract



Highlights

- *Poly*(allylamine) covalently modified with chlorophyllide *b* polymer is very stable in time and presents high fluorescent emission.
- The polymer formed a hydrogel when treated with CO_2.
- The formation of carbamate group was evidenced through *ss*-NMR and Raman experiments.
- Fluorescence emission at 664 nm as function of carbon dioxide concentrations shows a linear regression.

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Abstract

The chlorophyllide *b* derivatized polyallylamine (PAAChl) was synthesized. The resulting fluorescent polymer presented a high emission quantum yield, very stable in time. The polymer formed a hydrogel when treated with carbon dioxide that was characterized by Raman spectroscopy and solid-state NMR (*ss*-NMR). The adsorption of CO_2 took place through the formation of carbamate. Absorbance and fluorescence based analytical studies were performed. The UV-vis spectra of PAAChl, after additions of CO_2, showed a growing absorption peak at 250 nm corresponding to carbamate formation. The emission of fluorescence at 664 nm as function of carbon dioxide concentrations showed a linear regression with a detection limit of 2 ppm and a quantification limit of 5 ppm. The capacity of a chemical sensor is described by sensibility, selectivity, stability; response time and regeneration were also analyzed.

Keywords: CO_2, fluorescence, chlorophyllide *b*, sensor, raman, *ss*-NMR

1 Introduction

There is a need to selectively detect and quantify CO_2 with reliable and simple methods of chemical analysis in diluted samples or complex mixtures to solve environmental or clinical problems [1]. A variety of sensors have been developed based on potentiometric measurements [2], mass spectrometry [3] , gas chromatography [3], and non-dispersive infrared spectroscopya [4]. Unfortunately, these methods require bulky and expensive equipment that is not easily accessible to the majority of the laboratories. In contrast, the methods based on the emission of fluorescence are simpler, show high sensitivity along with a rapid response and can be used for small-sized sensors [5].

Nowadays, the development of functional materials together with the growing knowledge of the photophysics of chromophores facilitate the application of stimuli-responsive materials able to detect optical changes to effectively design sensors, probes and information displays [1,6]. In the case of optical sensors to carbon dioxide, most of the devices rely on the pH change induced by the solvation of CO_2 to carbonic acid, and therefore suffer from low selectivity [7,4,8]. However, some interesting developments rely on the combination of a colorimetric response to the pH change combined with a fluorescence measurement [8]. In this line of research, the contribution of Amao Y. [9] consistent with the colorimetric change of α-naphtholphthalein and the

measurement of fluorescence of tetraphenylporphyrin is worth emphasizing. Afterwards, Oter *et al.* [10] improved these experiments using ionic liquids (ILs) as optical sensors matrix materials.
Recently, a very selective sensor has been described, by combining the fluorescent imidazolium cations with primary amines that react with CO_2 to form imidazolium carboxylate [11].

The polyallylamine (PAA) contains polyethylene chains with pendant aminomethyl groups on alternating carbon atoms. It can be converted into a macroporous hydrogel through bubbling of CO_2 into their solutions with the subsequent formation of carbamates. The reaction connects the polymer chains to each other permanently, making a more rigid material that was explored by static and dynamic fluorescence measurements of an added pH sensitive luminophore: 2,6-naphthalenedicarboxylic acid (2,6-NDCA) [12]. We have obtained a fluorescent polymer with high emission quantum yield and described the polyallylamine PAA covalently modified with Chlorophyllide *b*, as the fluorophore moiety [13].

In this paper, we report the reaction of carbon dioxide with polyallylamine derivatized with chlorophyllide *b* (PAAChl) to generate a hydrogel with a very stable fluorescent response dependent on the amount of CO_2. The solid-state nuclear magnetic resonance (*ss*-NMR) of the functional PAAChl-CO_2 and PAA-CO_2 gels are compared with the corresponding fluorescent responses in order to understand the structural changes produced. Our results are very promising to obtain a selective and sensitive CO_2 sensor.

2 Experimental

2.1 Reagents

Analytical grade *poly*(allylamine hydrochloride), PAA, (Aldrich) was used as supplied. Liquids for gel preparations (reagent grade from Aldrich) were used without further purification.

Methyl chlorophyllide *b* was obtained from the methanolic extraction of spinach leaves following the procedure described by Starin and co-workers [14], without the step of sucrose separation. A column of silica gel 60 (0.063-0.2000mm) with a gradient mobile phase, varying the proportions of dichloromethane and methanol (0.5%, 1.0%, 1.5%, 2.0% and 8%) was used in the purification step obtaining a 95-98% of chlorophyllide b in the solid extract.

2.2 Apparatus

A HP8452 diode array spectrophotometer and a quartz crystal cell were used to obtain the UV spectra. FTIR spectra were recorded with a Thermo Nicolet 380 Thermo-Scientific.

The fluorescence measurements were carried out with a Shimadzu RF-540spectrometer. The samples were examined with an Olympus FV300 confocal microscope (Model BX61) with acquisition software FluoView3.3. The analysis of the confocal images and the co-localization was performed using image analysis software Image - Pro Plus version 5.1.2 (Media Cybernetics, USA). All images were obtained with a cooled CCD camera.

Raman scattering spectra were collected on a Renishaw in-ViaReflex confocal microspectrometer, equipped with a CCD detector of 1024 × 256 pixels, an holographic grating of 2400 groves/mm, and a 50-mW Ar laser (514 nm and 785 nm wavelength) as the excitation source. Spectra were measured in the 100–1600 and 2600-3300 cm^{-1} Raman shift region at 1 cm^{-1} spectral resolution. Measurements were carried out in conditions of high confocality (3 pixels of the CCD detector and 20 µm slit width) through a 100 × Leica metallurgical objective (numerical aperture of 0.9), which limits the diameter of the laser beam to about 1 µm.

High-resolution ^{13}C solid-state spectra for the polymers were recorded using the ramp ^{1}H-^{13}C CP-MAS sequence (cross-polarization and magic angle spinning) with proton decoupling during acquisition. All the *ss*-NMR experiments were performed at room temperature in a Bruker Avance II-300 spectrometer equipped with a 4-mm MAS probe. The operating frequency for protons and carbons was 300.13 and 75.46 MHz, respectively. Glycine was used as an external reference for the ^{13}C spectra and to set the Hartmann-Hahn matching condition in the cross-polarization experiments in ^{13}C spectra. The recycling time was 4 s and the contact time was 1.5 ms. The spinning rate for all the samples was 10 kHz. The SPINAL64 sequence (small phase incremental alternation with 64 steps) was used for heteronuclear decoupling during acquisition with a proton field H_{1H} satisfying $\omega_{1H}/2\pi = \gamma_H H_{1H}$ = 62 kHz [15]. The proton spin-spin relaxation times (T_2^H) and 2D ^{1}H-^{13}C Wideline Separation Experiment (2D WISE) were performed as in previous works [16].

The PAAChl-CO_2 was analyzed by ^{1}H HR-MAS NMR by packing the sample swelled with D_2O into a 4-mm ZrO_2 HR-MAS rotor with a 50 µL spherical insert and the sample was spun at 4 kHz.

2.3 Preparation of polyallylamine-chlorophyllide b (PAAChl)

A solution of chlorophyllide mixture (20 mg) extracted from spinach, in dichloromethane (1 mL), was added to a solution of PAA (15 mg) in dry methanol and 0.04mL of tri-ethylamine (TEA) [13]. The mixture was stirred for 24 h in darkness at room temperature. Subsequently, an excess of sodium borohydride was added after cooling the solution in an ice bath and stirring continued for 1 h. Methanol was removed under reduced pressure and the aqueous solution was then purified by dialysis against MilliQ water in regenerated cellulose acetate membranes with a 12,000 Da cut-off. A solid modified polymer containing 2.5×10^{-3} mg/mg of chlorophyllide was obtained after lyophilization. When not in use, PAAChl was stored at 4-8ºC.

2.4 Preparation of gels

A N_2-saturated methanolic solution of 1 mL of 2.9 M NaOH was added dropwise to a N_2-saturated suspension of PAAChl (6.0 mg) in 5 mL of methanol. The mixture was stirred for 3 h under an N_2 atmosphere. The mixture was centrifuged and the solution was separated. Gels were prepared by bubbling dry CO_2 through the PAAChl solution for 5 min at a rate of 20 cc/min.

2.5 Analytical Measurements

CO_2 was generated anaerobically by dropwise addition of degassed HCl 2M to solid Na_2CO_3. The so produced CO_2 was bubbled into degassed methanol in order to get a saturated solution ($[CO_2]$ = 37mM) [17].

Absorbance and fluorescence-based analytical studies were performed adding successive aliquots (10µL) of the saturated methanolic solution of CO_2 (37mM) to N_2-saturated solution of PAAChl (1.0 mg) in 3 mL of methanol. The mixture was gently stirred with a magnetic bar during the assays.

3 Results

The phenomenon of reversible association/dissociation of polyallylamine (PAA) by cross-linking/uncross-linking in response to CO_2 bubbling (Scheme 1) has been widely studied [12]. Following this premise, the formation of a hydrogel polymer of PAAChl with CO_2 in methanolic solutions was evaluated.

<Scheme 1>

Firstly, amino groups of PAA were derivatized with chlorin core of chlorophyllide *b* (Scheme 2), yielding a green polymer with two intense absorption bands in methanolic solution, one at 402 nm (Soret band) and another one at 660 nm, characteristic of the chlorin aromatic structure [13].

<Scheme 2>

Moreover, this material shows a very intense fluorescence band at 664 nm when excited at 400 nm, exactly as the chlorophyll molecule (Fig. 1) [18].

<Figure 1>

Consequently, the methanolic solution of the PAAChl showed light absorption in the orange and red part of the spectrum and an equivalent orange or red fluorescing. Later, the treatment of this green polymer dissolved in methanol with CO_2 leaded to a gel that also showed similar qualitative fluorescent characteristic. Fluorescence microscopy images of PAAChl-CO_2 are depicted in Figure 2, in which the gel was excited at 545-580 nm (A) and 460-490 nm (B), respectively. There is a correspondence between the fluorescence excitation and emission spectra obtained in methanolic solution (Fig. 1) and the colors observed in the images (Fig. 2) where the chlorophyllide *b* molecules were spread in the polymer material rendering a homogenous distribution of the fluorescence.

<Figure 2>

3.1 Raman analysis

The solid polymers were studied by Raman spectroscopy with 785-nm laser excitation in order to minimize the chlorophyllide fluorescence. The PAA spectrum (Fig.3 A) is consistent with the -$[CH_2CH(CH_2NH_2)]_n$- structure, while the samples treated with CO_2 (Fig. 3 B and C) showed also a very intense band at 1060 cm^{-1}, corresponding to the carbamate formation [19,20] together with a weak band at 227 cm^{-1} [21]. In spite of the intense fluorescence of the chlorophyllide ring, some small signal in the low-frequency region (179, 704 and 1431 cm^{-1}) corresponding to this molecule can be observed for PAAChl-CO_2 (Fig 3. C) [22–24]. Figure 3 E, D and F show the Raman scattering spectra in the ν(C-H) region for the three different polymers (PAA, PAA-CO_2 and PAAChl-CO_2). The bands at 2971 and 2917 cm^{-1} are straightforwardly assigned to the methylene antisymmetric ($\nu_a(CH_2)$) and the signal at 2872 cm^{-1} to the symmetric ($\nu_s(CH_2)$) stretching modes [17-19]. As expected, the hydrogel showed a lower symmetric mode (~2870 cm^{-1}) due to the diminution of polymer chain mobility. It is

noteworthy that not only PAAChl (not shown), but also PAAChl-CO_2 (Fig. 3 C and F) showed a flat Raman spectrum due to the emission of fluorescence of the chlorin portion.

<Figure 3>

3.2 Solid-state NMR studies

Solid-state nuclear magnetic resonance experiments were used to study the gelation process of the polymers under CO_2 saturated solutions. The ^{13}C CP-MAS spectrum for the PAA polymer presents the characteristic carbon resonance signals at 31.5, 35.2 and 43.7 ppm assigned to the -**C**H_2-, -**C**H< and -**C**H_2-NH_2, respectively (Fig. 4A). The amount of chlorophyllide in the polymer is very low (0.25%) to be detected in a ^{13}C CP-MAS experiment (Fig. 4B), but is confirmed by UV-spectroscopy (Fig. 1B). A new ss-NMR signal at 164.9 ppm appears during gelation of PAAChl-CO_2 material, that corresponds to the carbon of the carbamate group (Scheme 1, Fig. 4C) demonstrating the functionalization of the PAA polymer with the molecules of CO_2. The narrow signal at 167.4 ppm was associated with the presence of some inorganic carbonate as a secondary product of the reaction of carbon dioxide with water during the synthesis of the gel in the PAAChl-CO_2 (signal 5, Fig. 4C). To confirm this analysis, the^{13}C CP-MAS spectrum of potassium carbonate was carried out showing a unique signal with a similar bandwidth at 168.6 ppm (Fig. 4D).

The high-resolution magic angle spinning (HRMAS) experiment has been successfully applied to hydrogels, rendering liquid-like ^1H-NMR spectra [26]. This technique was applied to PAAChl-CO_2 gels, showing the swelling behavior of this material upon the addition of deuterium oxide (D_2O) at a spinning rate of 4 kHz averaging the ^1H-^1H dipolar interactions at the static condition (Fig. 4E and F). Different signals were present at 1.63, 2.11, 3.17 and 7.79 ppm corresponding to the -C**H_2**- (H_1), -C**H**< (H_2), -C**H_2**-N- (H_3) and -N**H_2**/-N**H**-CO_2- respectively; however, it was not possible to detect signal corresponding to the chlorophyllide structure bounded to the PAA.

<Figure 4>

Since the carbon resonance signals of the chlorophyllide structure cannot be detected by *ss*-NMR due to the low amount of molecules bonded to the PAA polymer together with the low sensitivity of these experiments, different proton relaxation times were measured: ^1H longitudinal relaxation time (T_1^H) and ^1H transverse relaxation time (T_2^H). Also, 2D WISE experiments were performed to obtain additional information about the

dynamics of the polymeric network in the different samples. The results are summarized in Table 1.

<Table 1>

The 2D WISE experiment is a simple method to measure the local mobility of polymers by correlating the proton lineshape with the carbon chemical shift [20]. The linewidth of the ^{1}H line reflects the nature of the dipolar interaction between the protons and thus can be used to monitor the mobility of the polymer chains. For the selected carbons signals (Table 1), the PAA and all the modified forms exhibit similar linewidth (67± 2 kHz) in the ^{1}H dimension. Similar ^{1}H linewidth from the 2D-WISE results are observed in different samples of *poly*(ethylenimine) polymers [16]. However, the relaxation times probed to bring additional dynamic information in polymer systems were both amorphous and crystalline domains are present.

From T_1^H and T_2^H values, it can be concluded that PAA has an ordered structure with strong interactions between the polymeric segments whereas the derivatized material (PAAChl-CO_2) is more complex due to the coexistence of both ordered (short T_2^H) and amorphous regions (large T_2^H) [16]. However, the PAA-CO_2 presents a T_2^H mono-exponential decay associated to a good dispersion of the carbamate groups due to a greater flexibility of the polymer network than the PAA (Table 1).

Interestingly, the polymeric structures present a single T_1^H that can be attributed to a fast exchange of water molecules between the bound, structured, and bulk water fractions [21-23]. This hypothesis can also explain the decrease of T_1^H for PAAChl-CO_2 and PAA-CO_2 considering that when the polymer chains dock with one and other, during gelation process, a portion of the hydration sheath is displaced. In the case of the PAAChl, the presence of the hydrophobic chlorophyllide moiety reinforces this effect together with a disorder or the polymeric chains due the chemical modification.

3.3 Absorbance and fluorescence-based analytical studies

Analogous effects were observed in the corresponding fluorescent emission spectra, where a decrease in the intensity of fluorescence with increasing concentration of CO_2 was observed. The quenching effect produced by the docking of polymer chains is linearly related to the concentration of carbon dioxide.

Therefore, in order to evaluate the sensing properties of the polymeric material, we obtained the UV-vis spectra of PAAChl after additions of CO_2. Figure 5 shows these results, where a growing peak at 250 nm, which was reported as characteristic of the

carbamate group [24,25], is observed with increasing concentration of CO_2. However, this band may be nonspecific for an analytical determination because a large number of compounds exhibit absorption in this region.

<Figure 5>

On the other hand, the fluorescence emission spectrum, by exciting at 400 nm, showed that the band at 664 nm decreases proportionally with the concentration of carbon dioxide (Fig. 6) with high specificity. This emission at 664 nm showed a linear range response to increasing concentrations of carbon dioxide up to 460 ppm (inset Fig. 6). The linear regression equation for the system is expressed as $A = -625\ ppm^{-1} x + 9.8\ 10^3\ ppm^{-1}$, R^2: 0.9928, the detection limit being of 2 ppm, the quantification limit 5 ppm and the sensitivity 625 ppm^{-1}.

<Figure 6>

The selectivity of this sensor is due to the carbamate formation between the amine groups of the PAA and the CO_2 added (Scheme 1) [12]. Therefore, volatile compounds which are expected to exist in the air environment, such as carbon monoxide, sulfur dioxide, nitrogen dioxide, hydrogen chloride, ammonia, dry air and water vapor, do not interfere in the determination. Moreover, the response time depends on the carbamate formation rate, which was estimated as ~10^3 M^{-1} s^{-1} [30–32]. And, as the reaction is reversible, the carbamate formed can be turned into PAAChl and HCO_3^- by lowering the pH or dissolving the hydrogel in water. Thus, it was possible to regenerate the PAAChl by dissolving the solid PAAChl-CO_2 in acidic water and lyophilizing.

It worth mentioning that the lifetime of the PAAChl was observed for 12 months after being synthesized. The results show good stability when storing the polymer methanolic or water solution at 4-8ºC. During this time, the chlorin ring suffers no deleterious effect, so the intensity of the fluorescent response does not vary.

4 Conclusions

We report the application of a fluorescent responsive chlorophyllide-hydrogel for carbon dioxide detection. The amount of fluorescent dye (chlorophyllide) is too small to be detected by *ss*-NMR, even though its presence can be inferred by the relaxation time T_1^H. The carbamate group can be evidenced through Raman, ^{13}C CP-MAS and UV-spectroscopy experiments. The material produces fluorescence at 664 nm with high quantum yield and the emission band is steadily declined by the addition of increasing concentration of CO_2. These results allow us to envisage the potential of

PAAChl for future applications as fluorescent polymer as the response time of the sensing, selectivity and regeneration of the sensor are based on the carbamate formation between the amine groups of the PAA and the CO_2.

Acknowledgements

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Vitae

Mariana Hamer obtained her PhD in Pharmacy from the University of Buenos Aires in 2014. She was postdoctoral at the Institute of Physical Chemistry of Materials, Environment and Energy from University of Buenos Aires, Argentina. She is currently a Teaching Assistant at the Department of Analytical Chemistry and Physicochemical in the School of Pharmacy at the University of Buenos Aires. She is a member of the Institute of Chemistry and Physical-Chemistry (IQUIFIB-CONICET) as researcher. Her area of research is the design of phases with molecular recognition ability to be applied to optical and electrochemical sensors.

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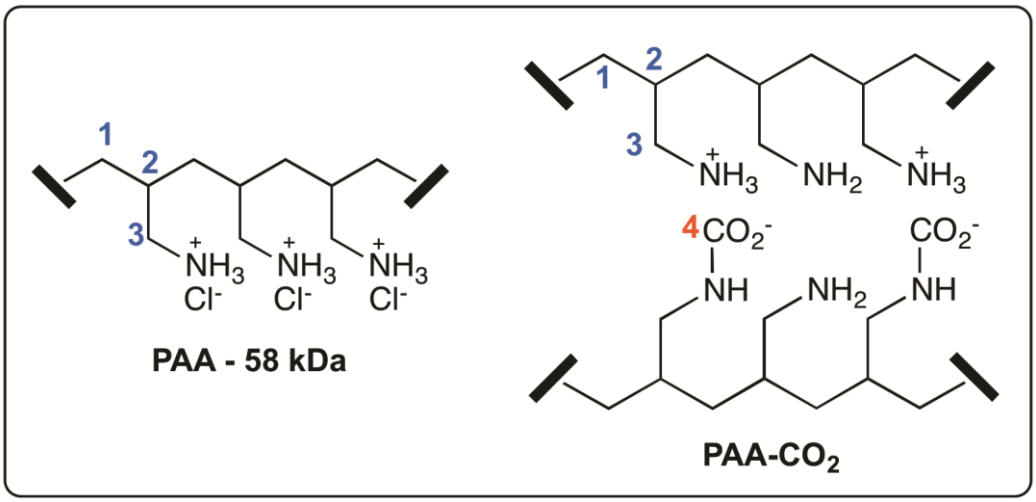
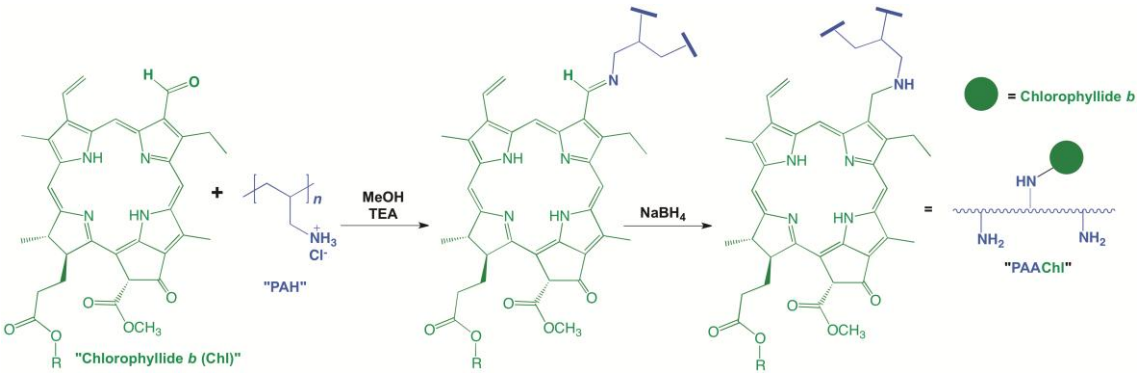


Scheme 1. Structure of the PAA-CO_2 after reaction of the PAA polymer with CO_2.The numbers indicate the assignments of ^{13}C CP-MAS, ^{1}H HR-MAS NMR signals in Figure 3.



Scheme 2. Synthesis of the PAAChl by initial formation of a Schiff base intermediate between PAA and the formyl group of chlorophyllide *b* (R = CH_3) and the posterior $NaBH_4$ reduction to amine.

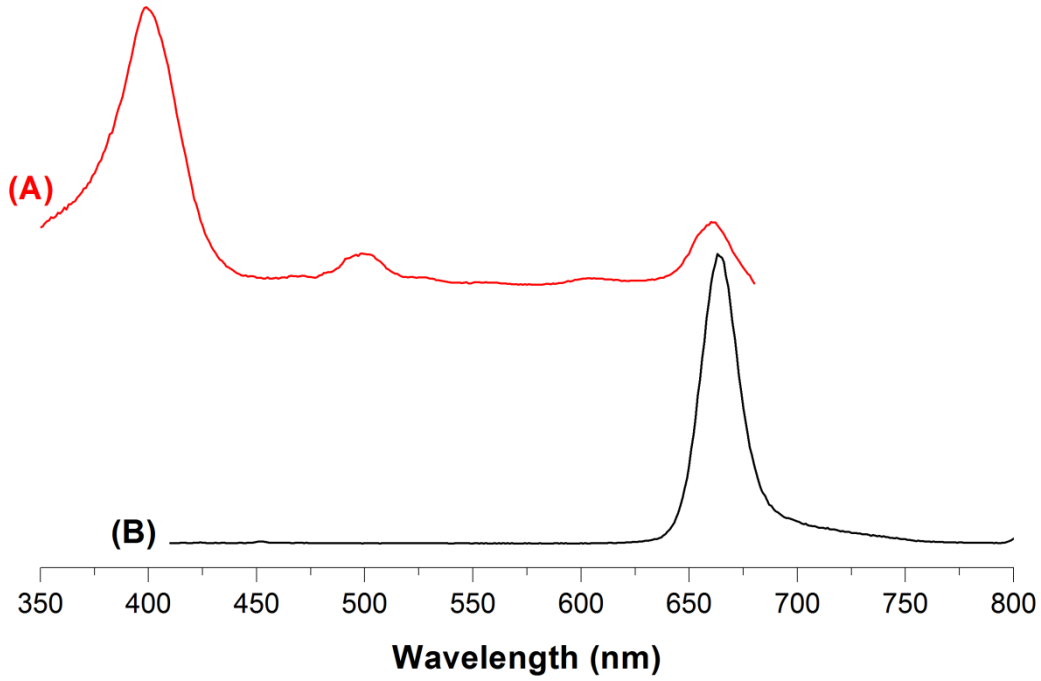


Figure 1. Excitation spectra with emission at 650 nm (A) and emission spectra with excitation at 400 nm (B).

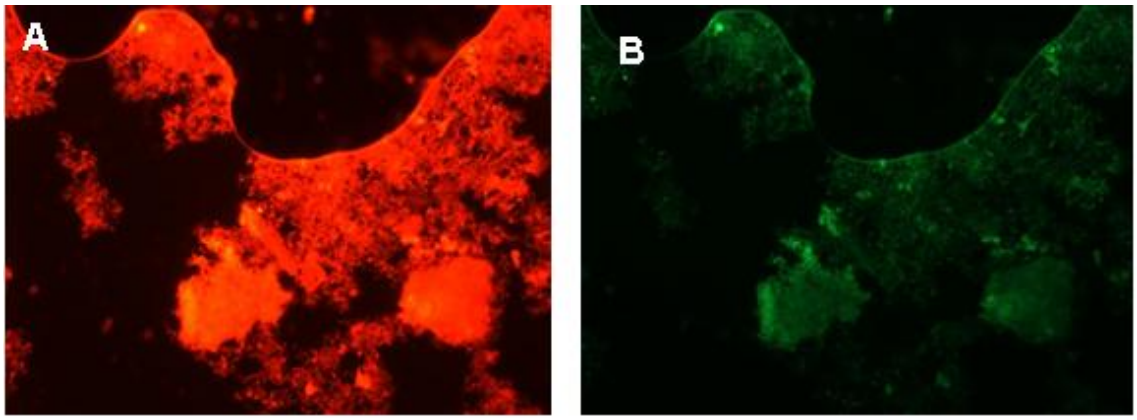


Figure 2. Images of fluorescence microscopy of PAAChl gel expanded in methanol (20X). Excitation at 545-580 nm and emission at 610 nm (A); excitation at 460-490 nm and emission at 510-550 nm (B).

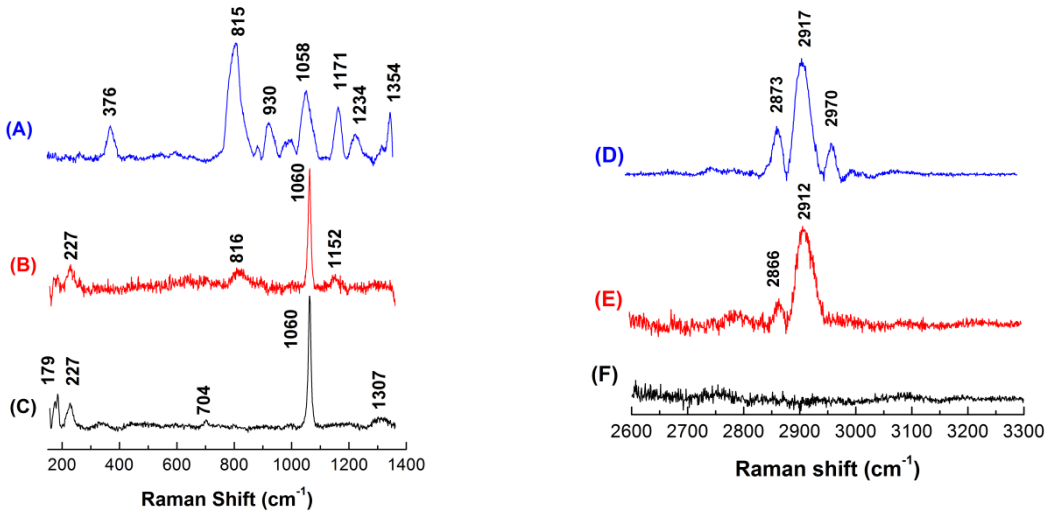


Figure 3. Raman spectra of solid PAA (A and D), PAAChl (B and E), PAAChl-CO_2 (C and F). Excitation wave-length: 785 nm.

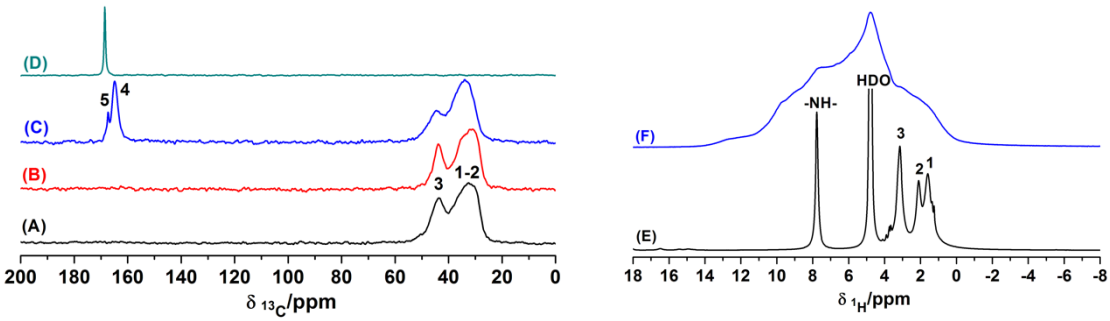


Figure 4. ^{13}C CP-MAS spectra for PAA (A), PAAChl (B), PAAChl-CO_2 (C) and potassium carbonate (D). ^{1}H HR-MAS NMR spectrum at spinning rate of 4 kHz (E) and static ^{1}H NMR spectrum for PAAChl-CO_2 (F). The numbering corresponds to those in Scheme 1.

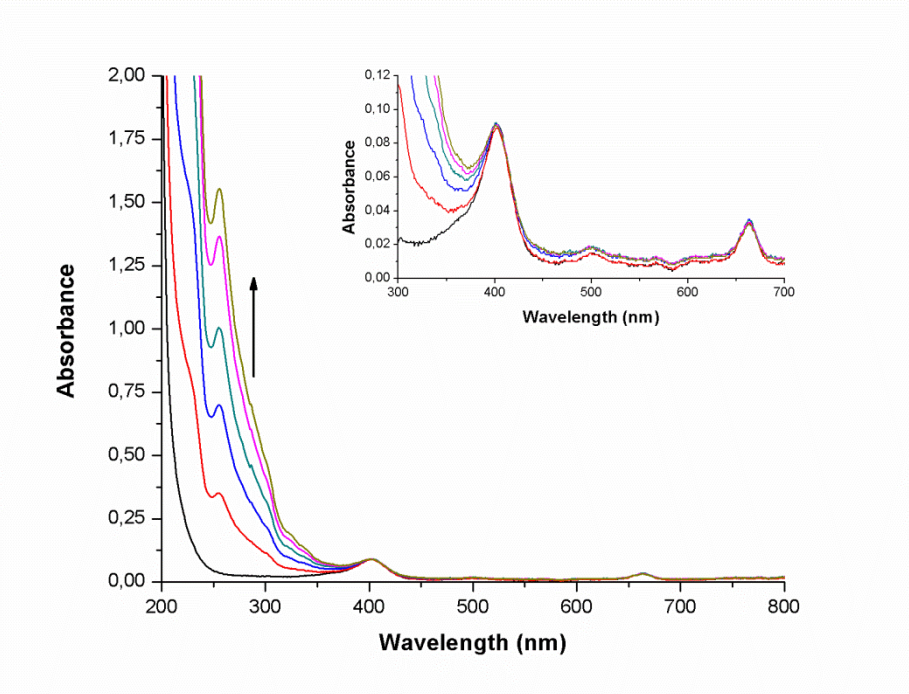


Figure 5. Absorption spectra of PAAChl after successive additions of CO_2.

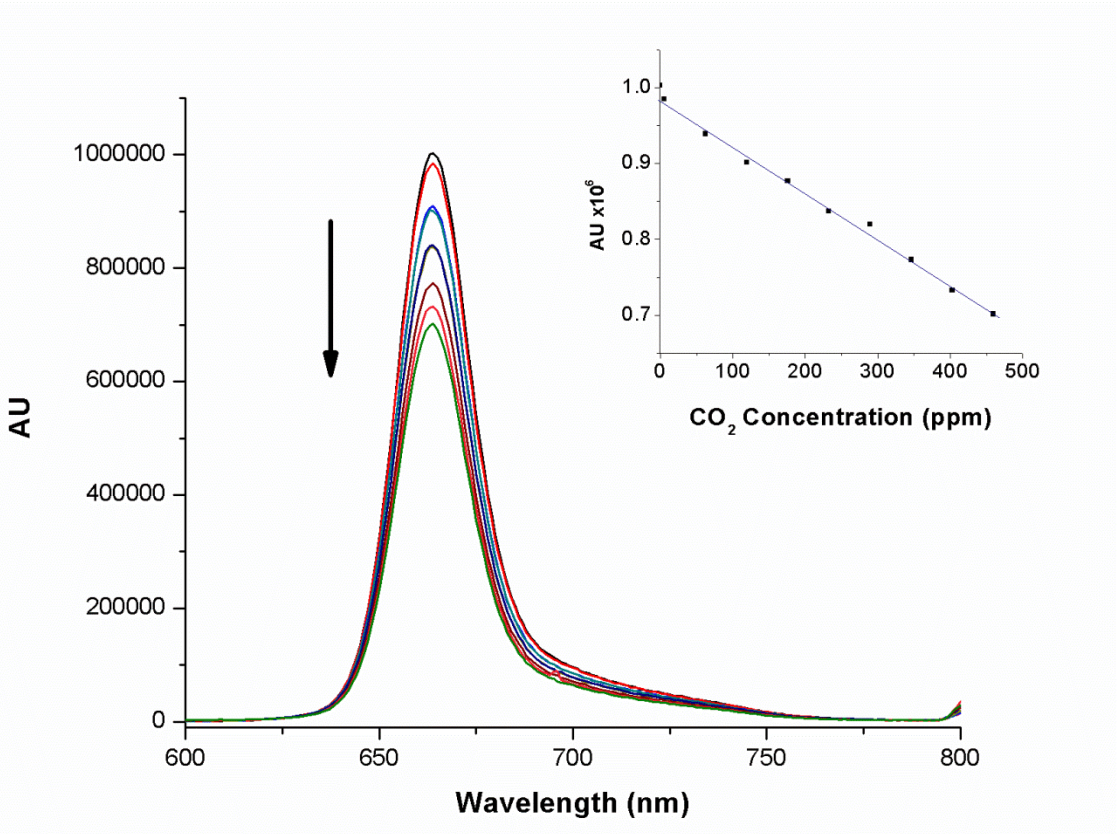


Figure 6. Changes in the fluorescence titration spectral features of PAAChl observed upon the addition of different volumes of CO_2 with excitation at 400 nm.

Tables

Table 1. T_1^H and T_2^H relaxation time values and 2D-WISE linewidth for the indicated ^{13}C chemical shift in each sample.

Material	T_1^H (ms)	T_2^H (μs)	2D-WISE ^1H linewidth (kHz)
PAA	710	30 (98)	67 (31.2 ppm)
		830 (2%)	66 (44.1 ppm)
PAA-CO_2	560	206 (100%)	69 (32.5 ppm)
			64 (42.9 ppm)
PAAChl-CO_2	281	50 (60%)	65 (29.9 ppm)
		990 (40%)	64 (43.3 ppm)

Graphical Abstract

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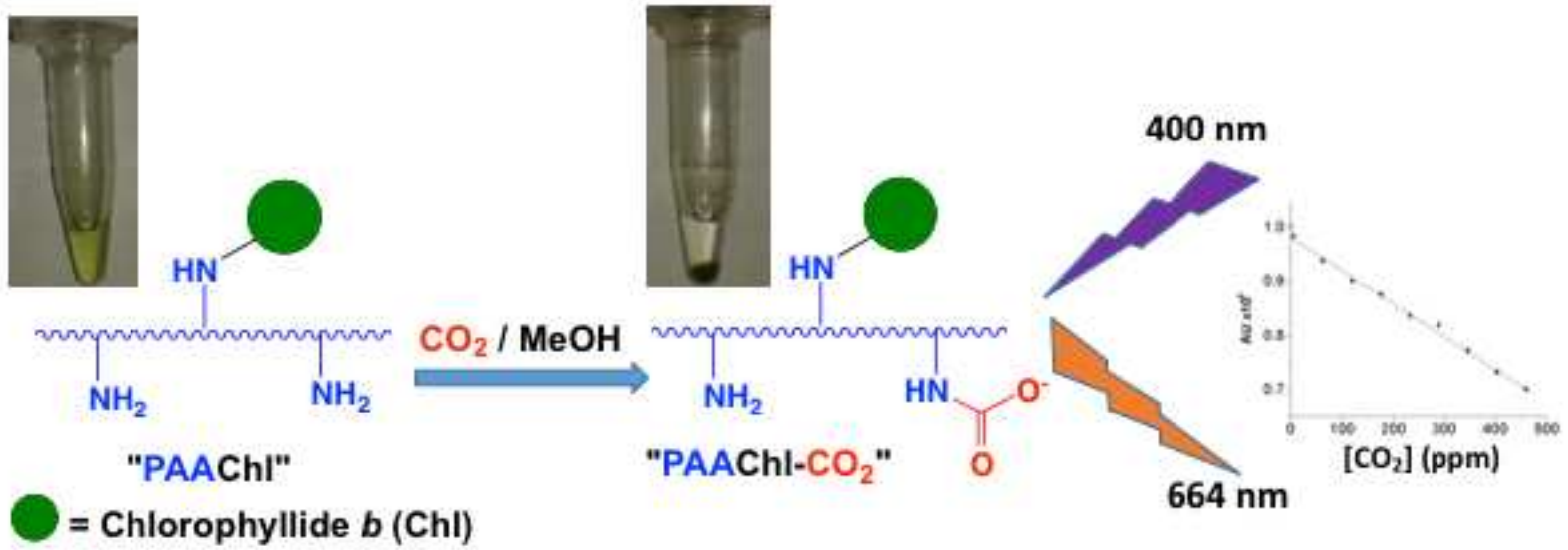

Scheme 1

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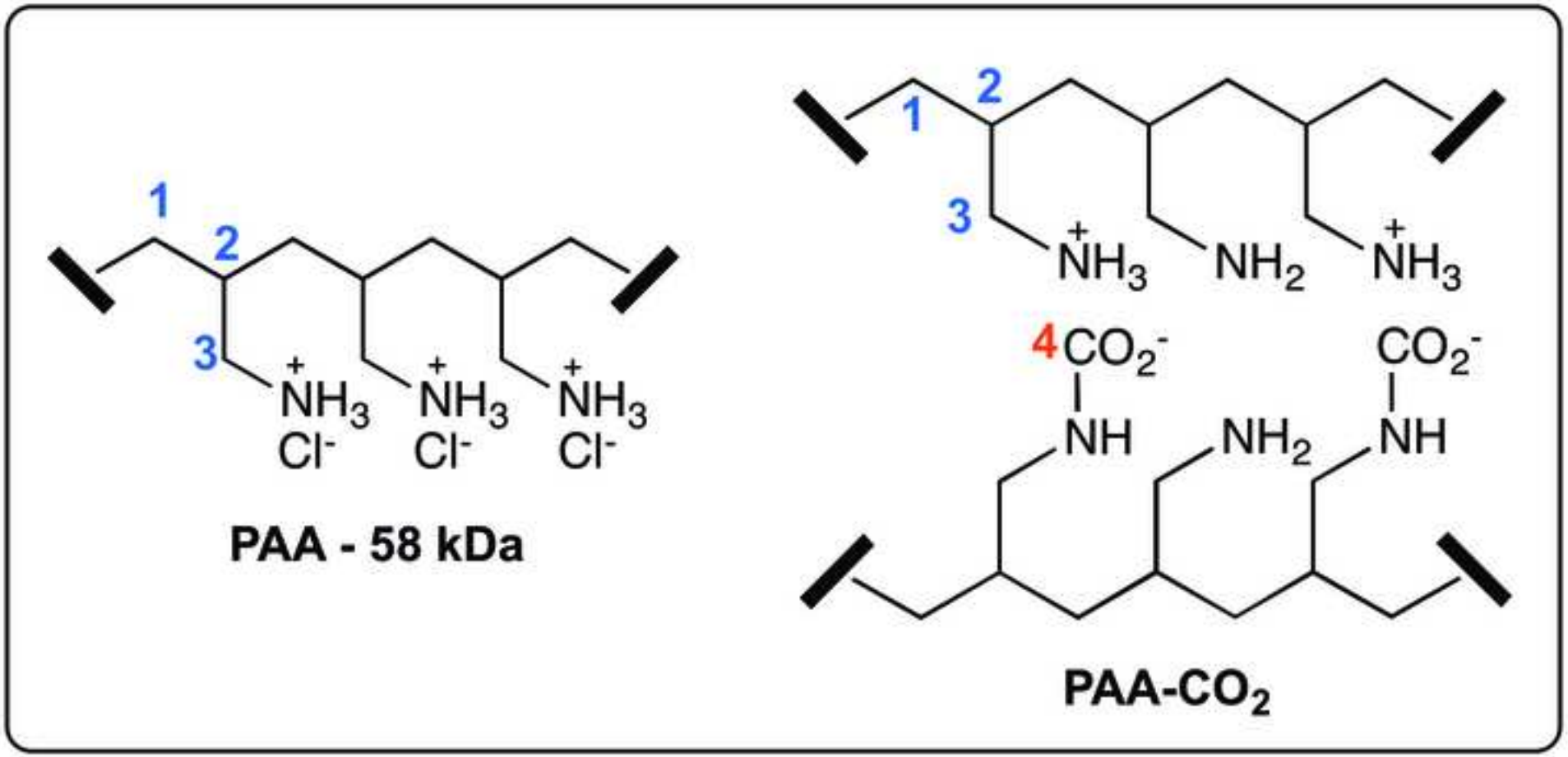

Scheme 2

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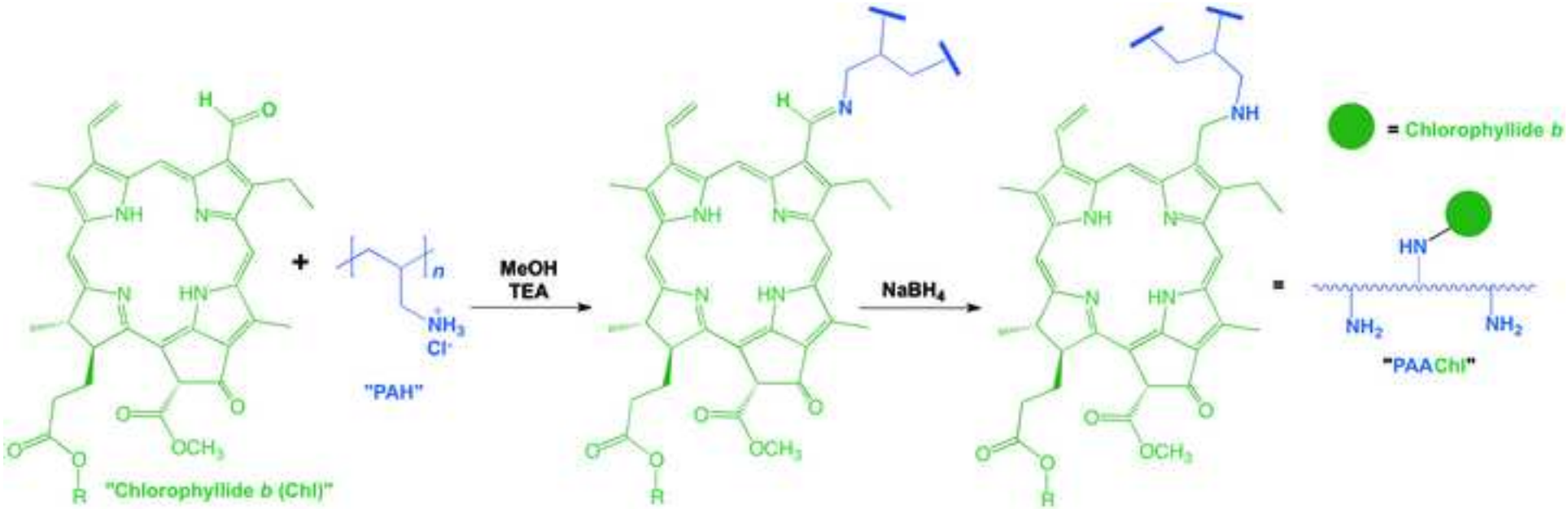

Figure 1

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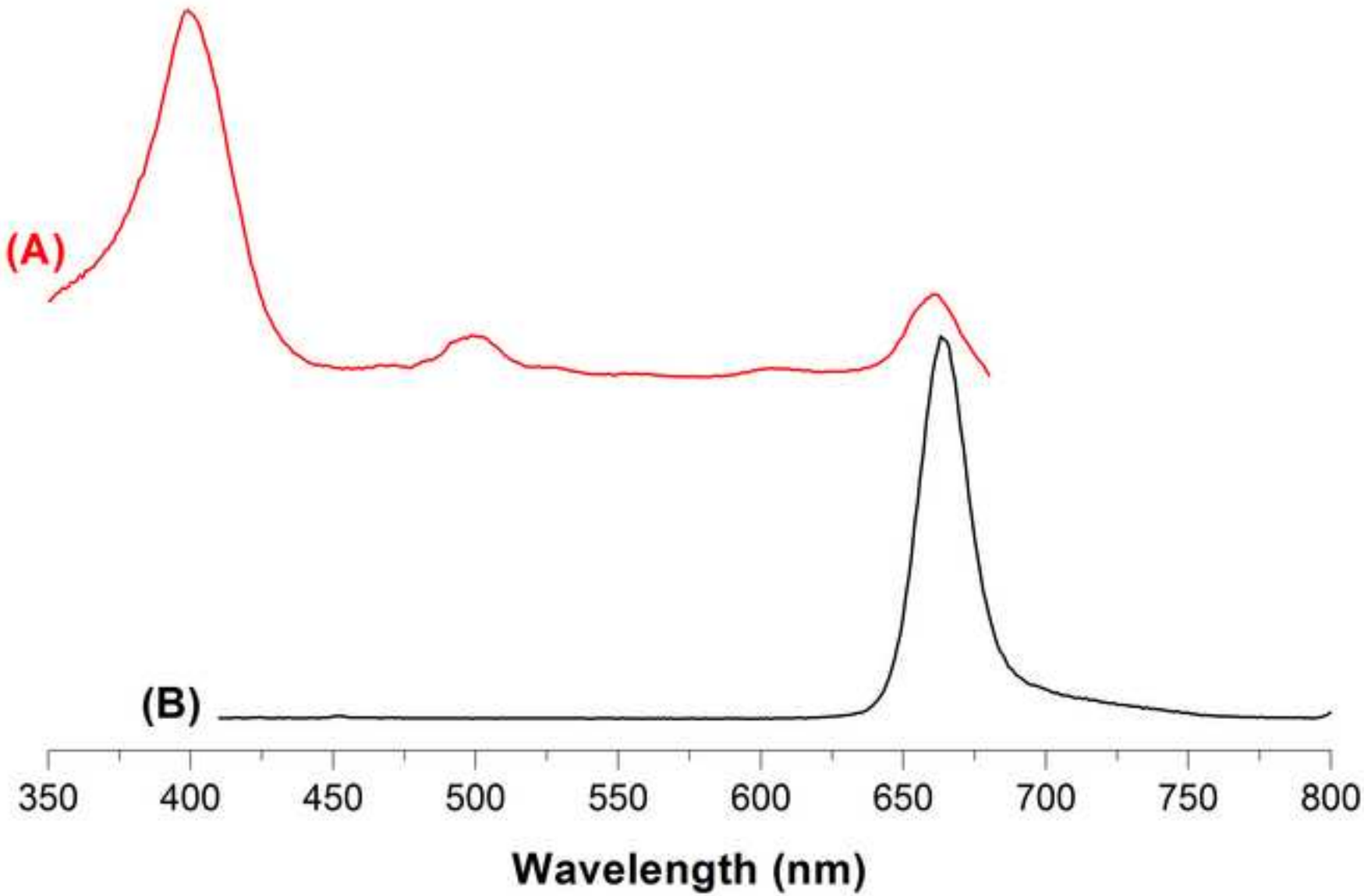

Figure 2

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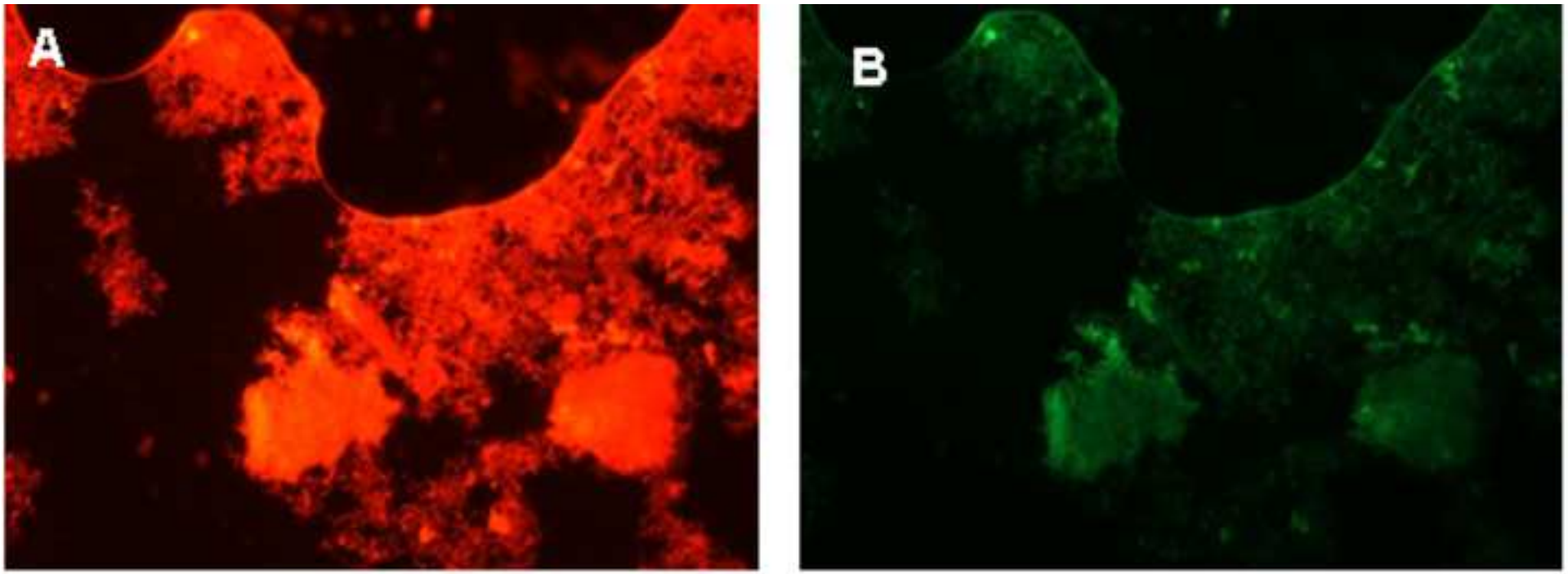

Figure 3a

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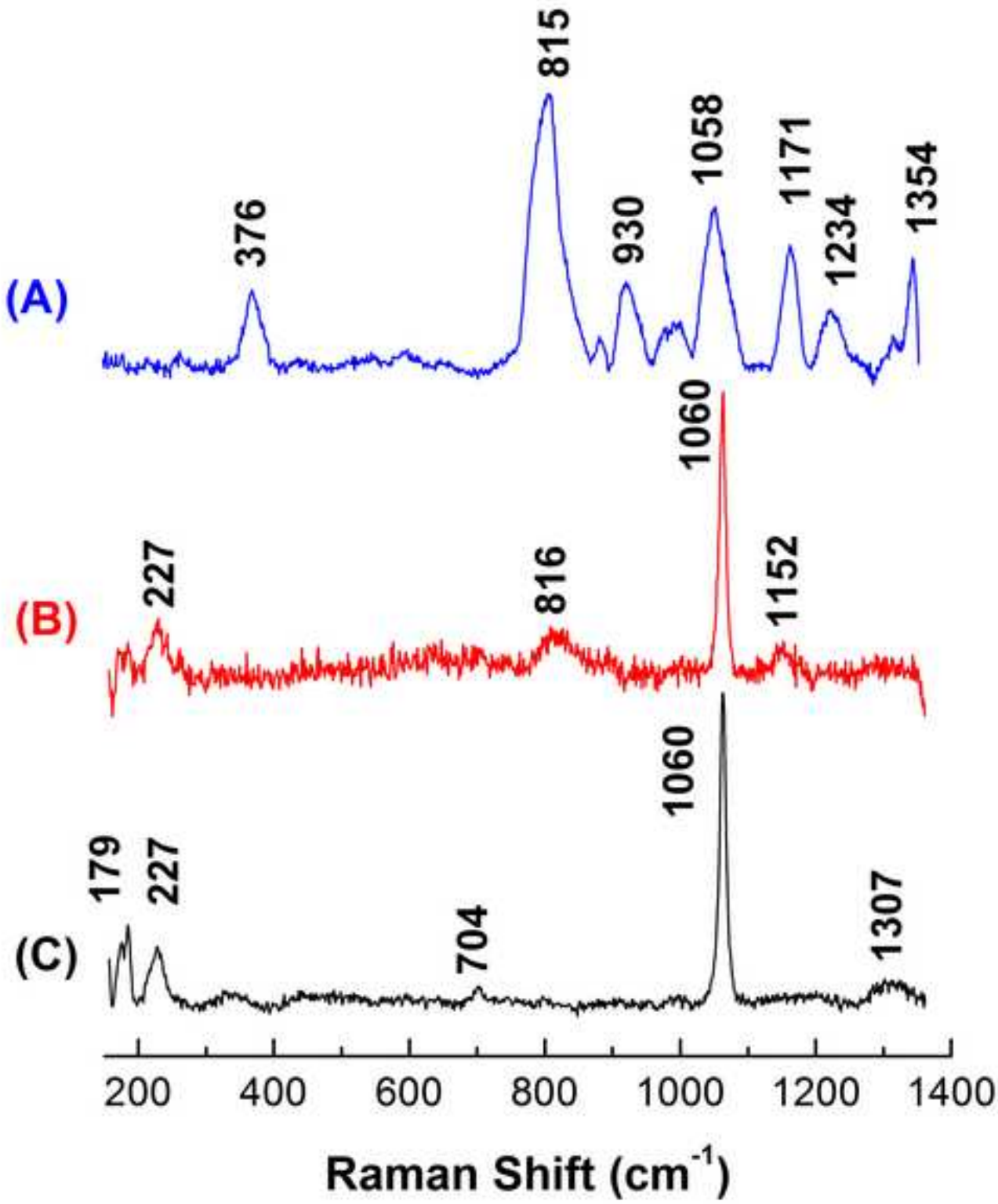

Figure 3b

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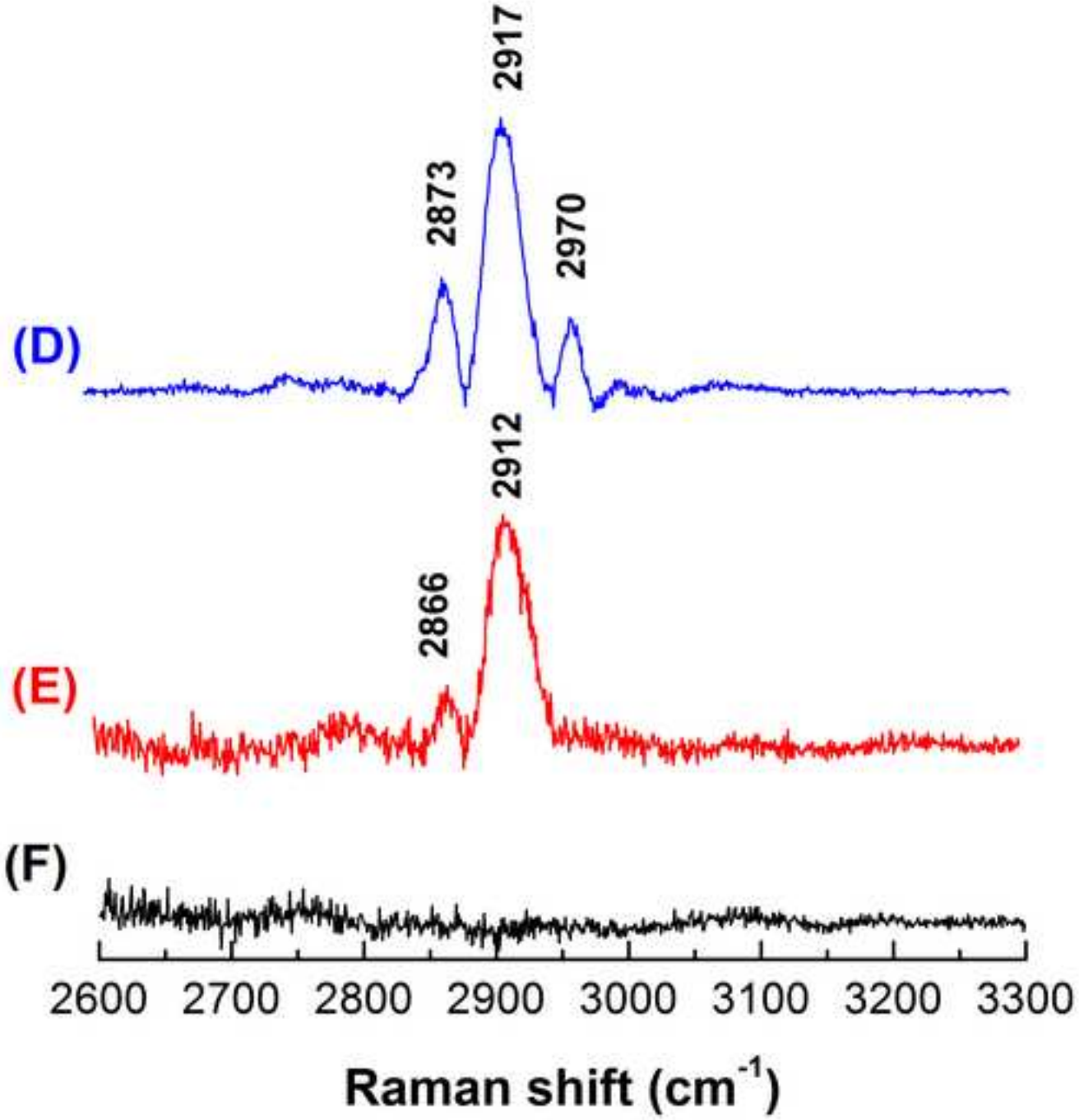

Figure 4a

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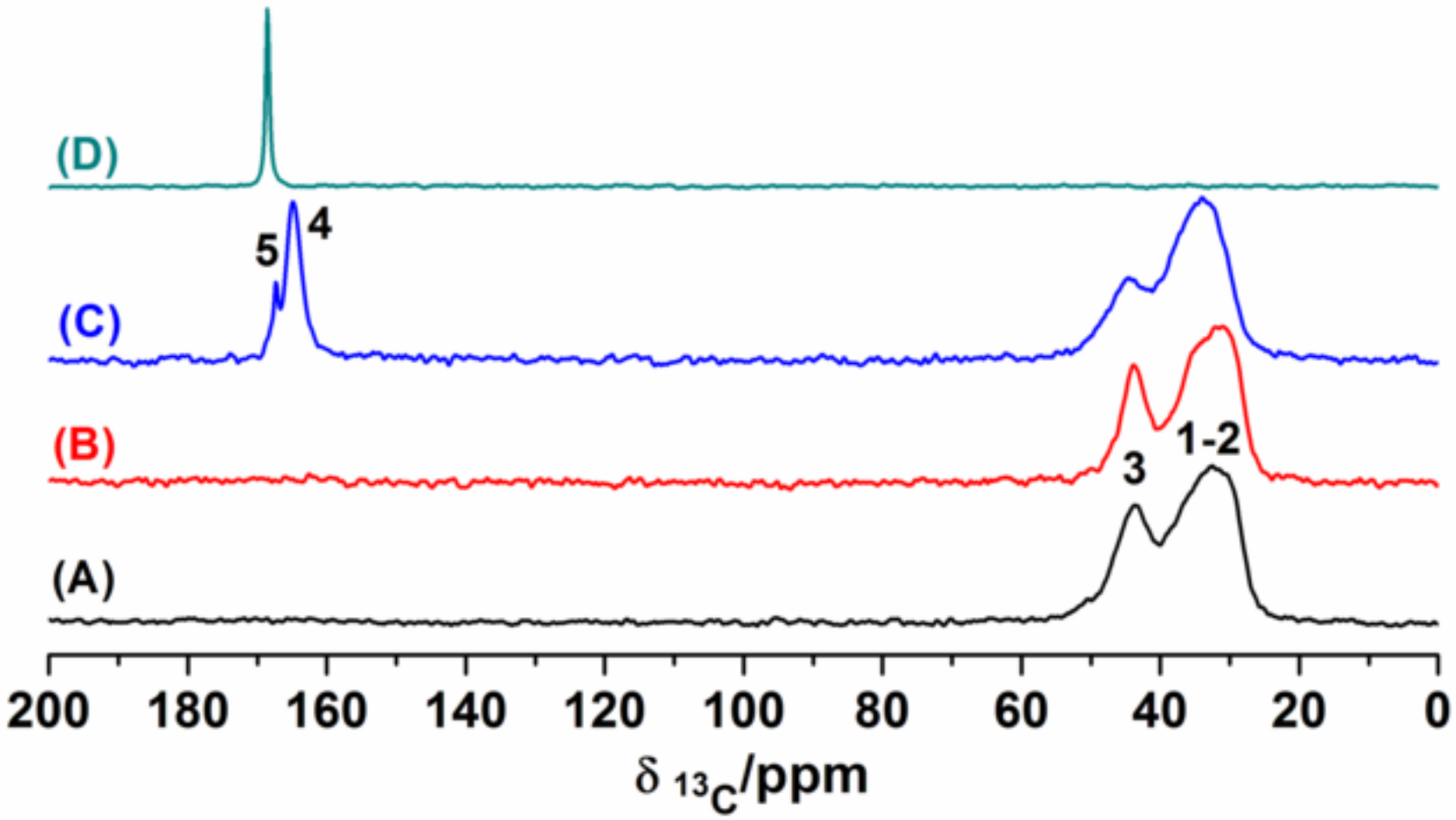

Figure 5

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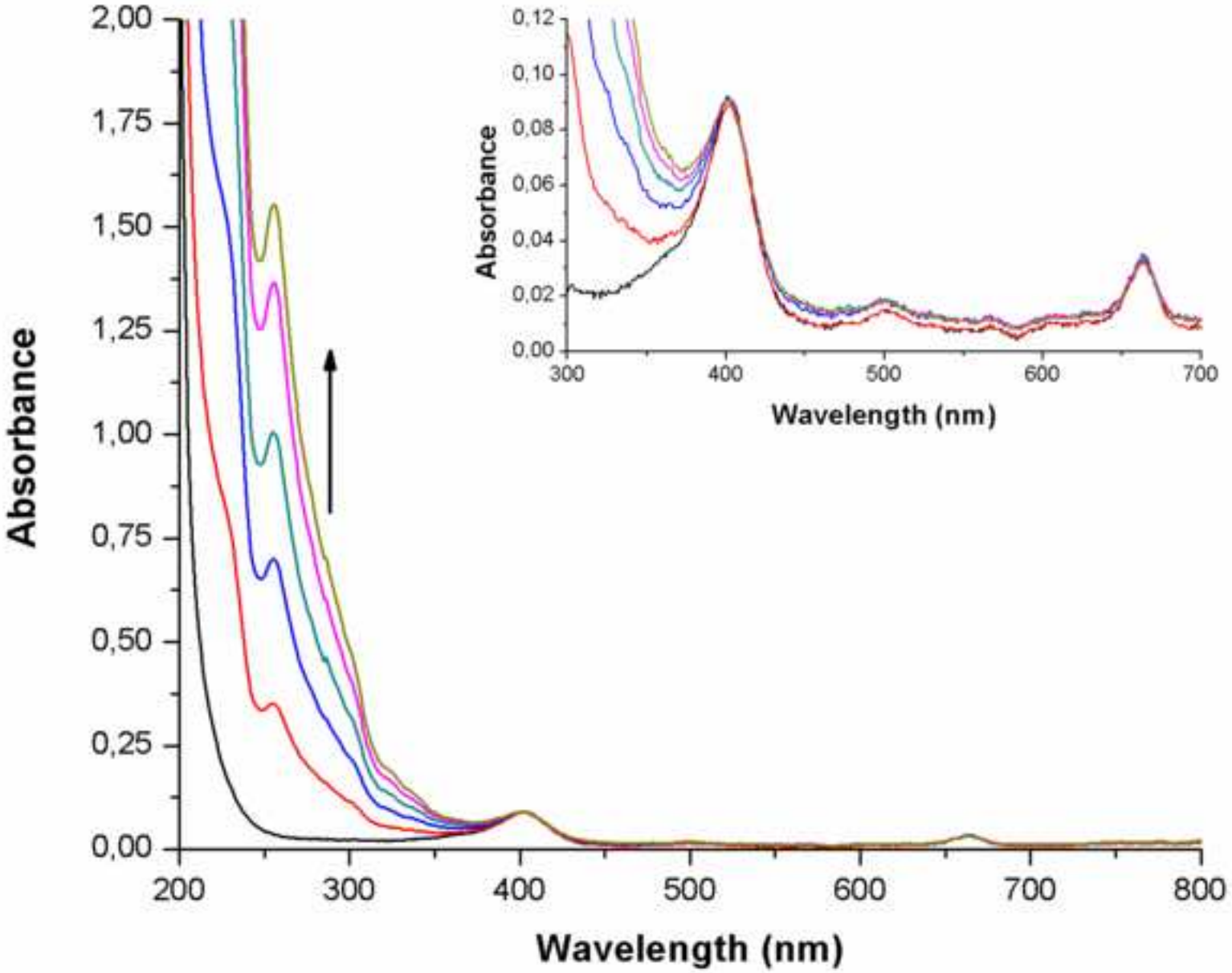

Figure 6

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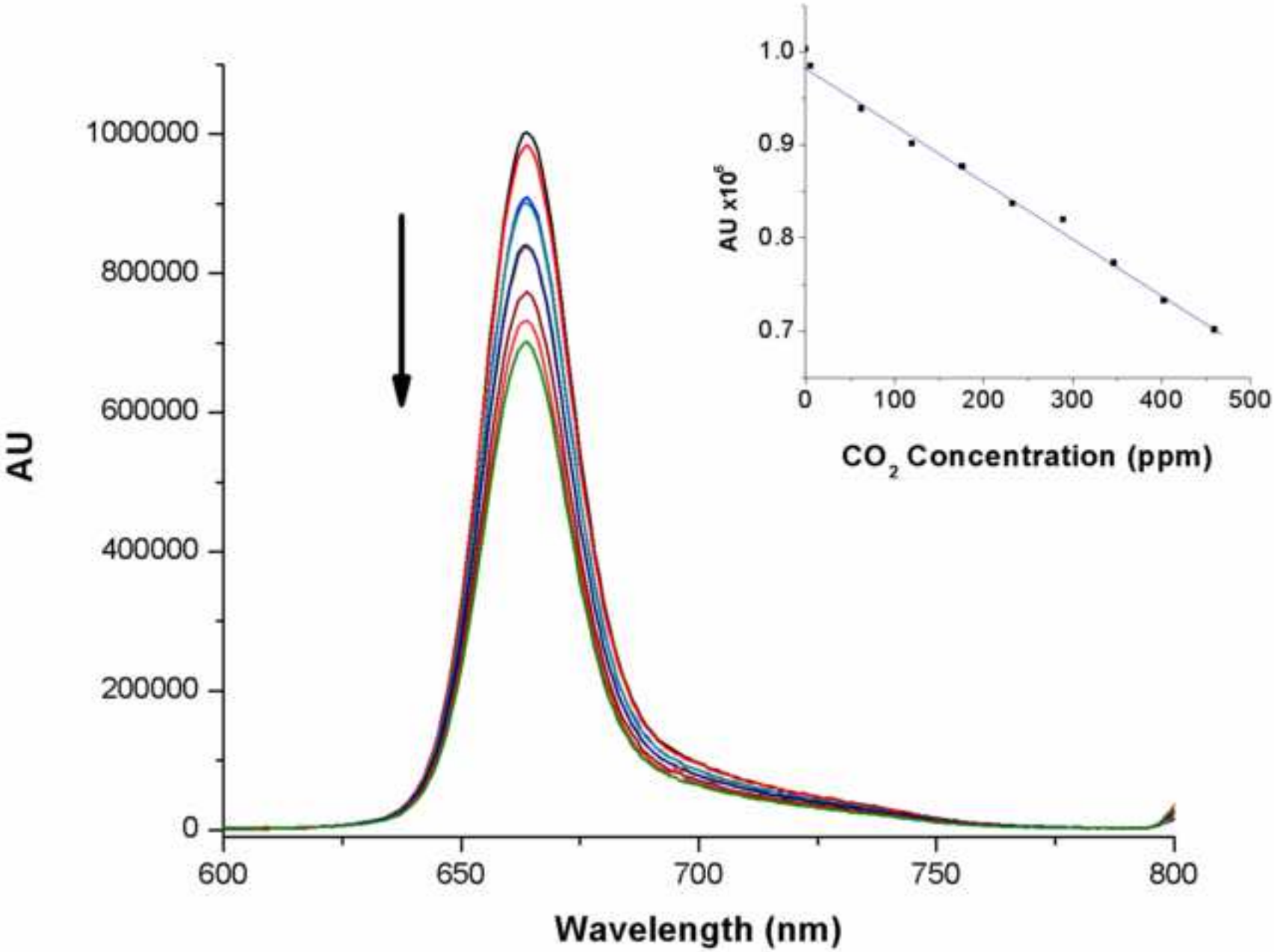

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***Author Biographies**

Mariana Hamer obtained her PhD in Pharmacy from the University of Buenos Aires in 2014. She was postdoctoral at the Institute of Physical Chemistry of Materials, Environment and Energy from University of Buenos Aires, Argentina. She is currently a Teaching Assistant at the Department of Analytical Chemistry and Physicochemical in the School of Pharmacy at the University of Buenos Aires. She is a member of the Institute of Chemistry and Physical-Chemistry (IQUIFIB-CONICET) as researcher. Her area of research is the design of phases with molecular recognition ability to be applied to optical and electrochemical sensors.

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