

Biological: Full-length

A new protocol to detect light elements in estuarine sediments by X-ray microanalysis (SEM/EDS)

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Abstract The analytical scanning electron microscope (SEM) has been used to determine the presence and distribution of atomic elements in mineralogy. However, the detection of light elements such as carbon is difficult to obtain with standard energy-dispersive X-ray spectrometry (EDS) and usual proceedings for SEM. This study proposes a new protocol to detect calcium carbonate by SEM/EDS using sediments from the Jaguaribe River estuary, NE Brazil, as a model. Handmade gold mounting discs (Au stubs) were used as sample support and samples were adhered with inexpensive glue (*Loctite Super_Bonder*) or directly disposed on the Au stubs. CaCO₃ and NaCl for chemical analysis were used as control and counterproof to the carbon adhesive tape. Control salts EDS analyses indicate that the method was efficient to detect light elements. Sediments obtained from different depths in the core sampled at the Jaguaribe River estuary consist of particles and aggregates with diverse morphology that covers a wide range of particle or aggregate size. Morphology and dimensions were similar for all core depths. Analysis of samples disposed on gold mounting disc without glue showed that sediment bulk particles usually presented small particles adhering on the surface. Clay minerals were predominant but silica was also often identified. Calcium was a trace element in a small number of sediment bulk particles. Biological and non-biological calcium carbonates, including nanoparticles, were identified in all core depths. X-ray emitted from Au stub did not interfere in the CaCO₃ EDS analysis. Calcium carbonate particles from sediments were identified using this novel approach.

Keywords sediment geochemistry, Jaguaribe River estuary, carbon, scanning electron microscopy/energy-dispersive spectrometry (SEM/EDS)

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Introduction

Greenhouse gases absorb infrared energy radiated from the earth, which results in an increase in the temperature of the troposphere. The concentrations of these gases—CO₂, CH₄, N₂O, chlorofluorocarbons—have been increasing in the atmosphere. Presently, the most important greenhouse gas is

CO₂ mainly due to the increase in the use of fossil fuels [1]. Interest in calcium carbonate formation and dissolution in the ocean has thus increased because of the central role these reactions play in the oceans and other aquatic systems response to the increasing partial pressure of carbon dioxide in the atmosphere. Reactions resulting in the formation and dissolution of calcium carbonate are also of central importance

to the sequestration of carbon dioxide in subsurface carbonate reservoirs and saline waters [2]. The components of the carbonate system in sediments have been traditionally characterized by simple gravimetric determination of calcium carbonate using a pre-weighed closed flask and the CO₂ evasion after HCl treatment of the sample [3] and, more recently, by methodologies such as video footage analyses to identify facies and biocoenoses classes of carbonate mounds [4]. On the other hand, from the geochemistry point of view, the carbonate mineralogy can provide much more important information about the nature of the sedimentary processes than simple concentration data and/or morphology.

Since 1970s, electron microscopy has been used in mineralogy and petrology [5]. Analytical electron microscopy has also proved to be an extremely useful tool to improve the spatial resolution for chemical analysis. X-ray microanalysis is an essential analytical approach to determine elemental concentration and distribution in mineralogical, geological and material research [6–9]. Large or bulk mineral specimens can be mounted on aluminium stubs with conductive paint or carbon tape and coated with 30-nm-thick carbon layer. However, X-ray production from the carbon tape and carbon coat as well as from the aluminium stub itself prevents an accurate detection of carbon and aluminium in an energy-dispersive X-ray spectrometry (EDS) analysis of small specimens [7,10].

The detection of chemical elements lighter than sodium by standard EDS and usual procedures for scanning electron microscopy (SEM) is not easy [7,11–13]. In addition, there are various ways in which an electron beam can adversely affect a sample during examination in an electron microscope, including heating, electrostatic charging, ionization damage, displacement damage and vibration [14]. In order to avoid vibration of specimens, especially delicate and small ones, they can be adhered onto the stub with white glue that is almost dry [10].

Carbon is a key element of the carbonate system [1,2,15], but the inherent difficulties of the analyses of light elements by SEM/EDS hamper the use of this powerful technique to interpret carbonate precipitation and accumulation mechanisms, in particular at complex matrixes such as marine and estuarine bottom sediments. Previously, conventional SEM/EDS

procedures were successfully used to characterize Jaguaribe River estuarine sediments [16], an environment where carbonate deposition plays a key role in regional carbon cycle [17]. In these sediments, the morphology of the particles typically varies from biological to non-biological origin, and biological to non-biological particle rates also vary according to sediment depth, probably reflecting past changes in regional climate and hydrology [18]. Silica oxides, aluminosilicates, sodium chloride and pyrite were predominant among the non-biological sedimentary particles. In these sediments, the morphology and elemental distribution of the particles have been carried out by standard proceedings for SEM and EDS; consequently, carbon detection is controversial due to the noise produced by the adhesive tape present in the preparation, making the full discussion of any process involved in the genesis of these particles difficult [16].

The main goal of this paper is to propose a new method to detect low-atomic-number chemical elements and their distribution, with emphasis on carbon, avoiding electron beam damage via EDS in SEM. For this, chemical analytical salts and Jaguaribe estuarine sediments were used as a model.

Materials and methods

Mounting disc

Discs—3 mm in diameter (Fig. 1a)—or fragments—10 × 10 × 0.5 mm (Fig. 1b)—of gold target [BALTEC (B 8010 072 21)] were adhered onto aluminium stubs with silver paint (Sigma) and used as sample support—Au stub. Adhesive cyanide acrylate ester [*Loctite Super_Bonder*[®] (LSB) glue] was dropped onto the Au stub at an angle of 45°, forming a thin film, probably a monolayer, in which lyophilized sediment particles were immediately attached to the Au stub (Fig. 1c) and dried at room temperature. Alternatively, lyophilized sediment particles were directly disposed on the Au stub (Fig. 1d). Au stub images (2.0 M) were taken with a SONY α-100 digital camera.

Controls and counterproof

Four controls were used: (i) Au stubs covered with LSB glue, (ii) calcium carbonate (MERCK—CX 0120) and sodium chloride (MERCK 1.06404) ad-

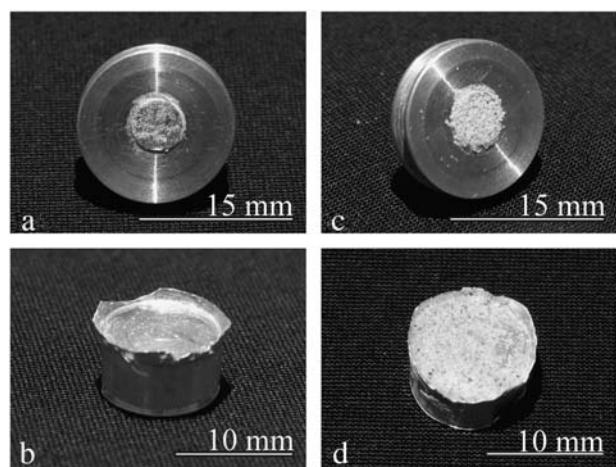


Fig. 1. Handmade gold mounting discs. (a) Gold disc (3 mm in diameter) and (b) fragments ($10 \times 10 \times 0.5$ mm) adhered onto aluminium stubs with silver paint. (c) Estuarine sediment particles adhered onto Au stub with LSB glue which was dropped on Au stub in an angle of 45° . (d) Estuarine sediment particles directly disposed on Au stub.

hered onto Au stubs with LSB glue and dried at room temperature, (iii) calcium carbonate and sodium chloride directly deposited on Au stubs, (iv) calcium carbonate and sodium chloride adhered onto carbon double-face adhesive tape as counterproof. Samples were not covered with carbon coating and were gently dried in an oven at 50°C for 24 h.

Sampling

Estuarine sediment samples were collected at the Jaguaribe River estuary, at the northeastern coast of Brazil, using PVC tubes (150×5 cm). Wet samples were collected from 5 to 10, 20 to 25, 55 to 60 and 65 to 70 cm depths in the core. These depths were selected based on their carbonate content, pre-determined by gravimetric method [3] and representing the lowest and highest carbonate concentrations. For analytical microscopy, 50 mg of lyophilized sub-samples from each selected depth were either adhered onto Au stubs with a thin film of LSB glue and dried at room temperature or deposited on the Au stubs without adhesive. Sub-samples were not covered with conductive coating and were maintained for 24 h at 50°C before analyses.

SEM/EDS

Morphological and analytical data were obtained using a SEM EVO 40 XVP ZEISS (Carl Zeiss SMT AG) with an energy-dispersive spectrometer IXRF

(IXRF Systems, Inc.) equipped with Si (Li) X-ray detector and ultrathin Be window (0.3μ) which had been calibrated with 99.9% carbon and aluminium standard. The microscope conditions for recording of the morphological information were: high vacuum mode, tungsten hot electron emission and acceleration voltage of 25 kV. For recording of the analytical signal for EDS, the conditions were: probe current varying between 500 pA and 1 nA, work distance of 8.5 mm, X-ray acquisition time of 90 s, X-ray exit angle of 35° and the same conditions described above. In addition, specimen holder temperature was controlled ($\sim 0^\circ\text{C}$) with a DEβEN (DEBEN UK Limited) coolstage accessory.

Results

SEM observations of large areas of the Au stubs covered with a thin film of LSB glue showed the striated relief of gold without discontinuities, but the adhesive film was not observed in backscattered electron (BSE) images or secondary electron (SE) images (Fig. 2a). EDS spectra revealed predominantly gold and the carbon peak was close to the background curve (Fig. 2b). Carbon could not be detected in the EDS element distribution maps (Fig. 2c), while gold was homogeneously distributed (Fig. 2d).

Calcium carbonate adhering to Au stubs with a thin film of LSB glue presented an irregular aggregated aspect in BSE images (Fig. 3a). EDS spectra revealed carbon, oxygen, calcium and gold (Fig. 3b). There was a clear overlap of carbon (Fig. 3c), oxygen (Fig. 3d) and calcium (Fig. 3e) in EDS element distribution maps. In contrast, carbon was not detected in areas among calcium carbonate aggregates in which there was a predominance of gold in the EDS distribution maps (Fig. 3f). Similar results were obtained for sodium chloride when analysed by this procedure for SEM/EDS.

Calcium carbonate directly disposed on the Au stubs without a thin film of LSB glue showed the same morphology described above. BSE images (Fig. 4a) and SE images of small grains ($<20 \mu\text{m}$) were analysed. EDS spectra revealed carbon, oxygen, calcium and gold (Fig. 4b). The overlapping of carbon (Fig. 4c), oxygen (Fig. 4d) and calcium (Fig. 4e) in the EDS element distribution maps was easily identified. There was a clear distinction between the calcium carbonate and gold (Fig. 4f) dis-

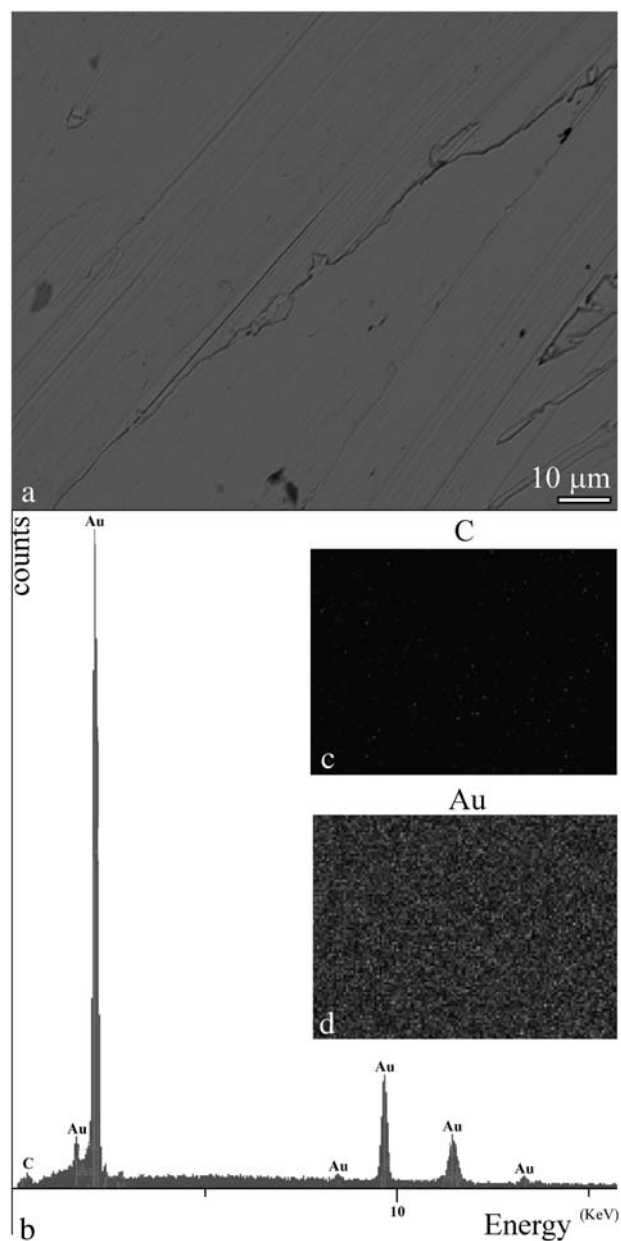


Fig. 2. (a) Handmade gold mounting disc (Au stub) covered with a film of LSB glue (SE image). (b) EDS spectrum of total area (in a) showing C peak, close to the white radiation, and Au peaks. (c) Elemental EDS map for C and Au (d). Carbon X-ray from glue was not detectable in its elemental map.

tribution. A similar procedure was successfully carried out with sodium chloride grains (Fig. 5). SE and BSE (Fig. 5a) images of sodium chloride bulk particles had a roughly cubic aspect. The overlapping of sodium (Fig. 5c) and chloride (Fig. 5d) in the EDS element distribution maps was observed.

In the counterproof, calcium carbonate aggregates adhering to carbon adhesive tape showed the same morphology described above (Fig. 6a). EDS spectra

revealed carbon, oxygen and calcium (Fig. 6b). The overlapping of carbon (Fig. 6c), oxygen (Fig. 6d) and calcium (Fig. 6e) in the EDS element distribution maps was easily identified. However, there was no

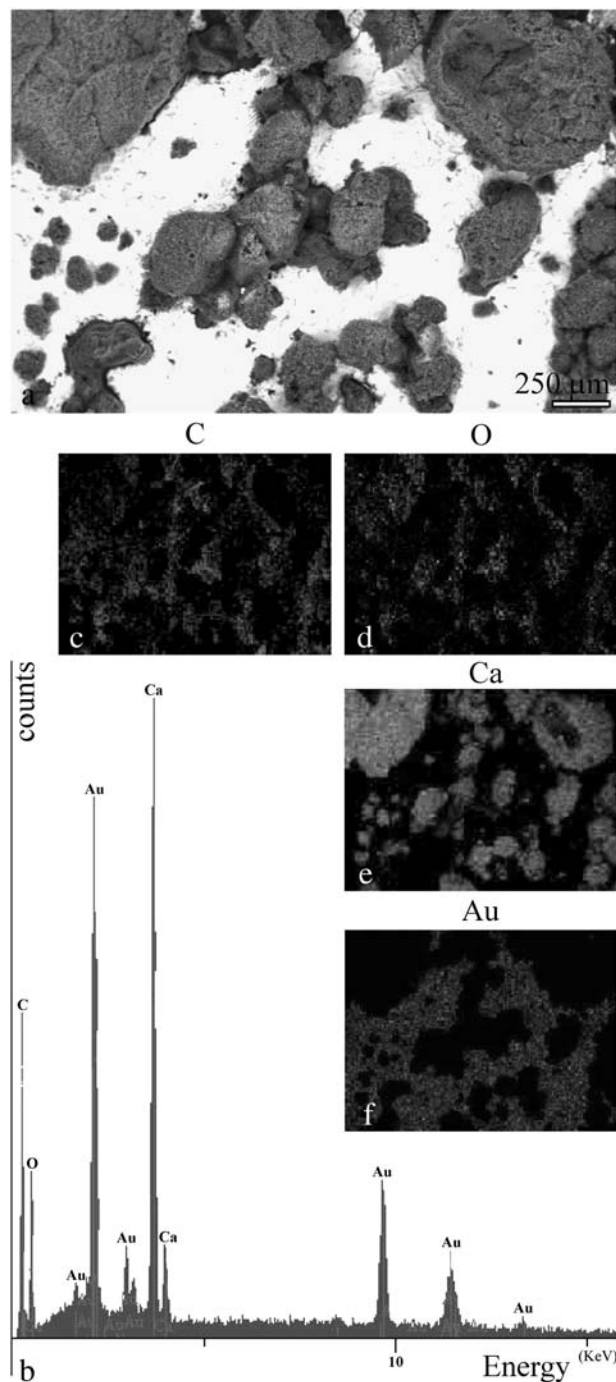


Fig. 3. (a) CaCO_3 adhered on gold disc with LSB glue show granulate aspect (BSE image). (b) EDS spectrum of total area (in a) revealing C, O, Ca and Au peaks. There is no overlap of peaks. Elemental EDS map for C (c), O (d), Ca (e) and Au (f). Note Au X-ray signal only from Au stub. Note a discrepancy between CaCO_3 fine particles distribution (a) and its carbon distribution map (c), probably due to strong contrast from Au stub.

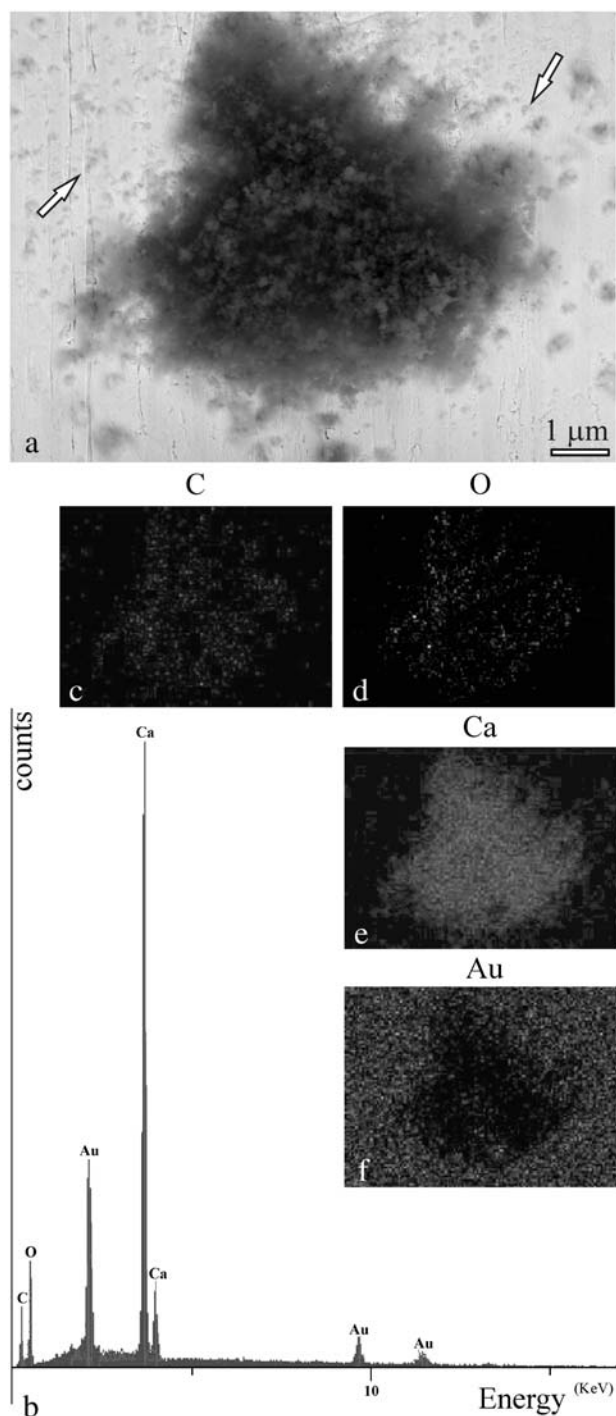


Fig. 4. (a) High magnification of CaCO_3 adhered onto Au stub with LSB glue (BSE image). Nanoparticles ($<1 \mu\text{m}$) are easily identified (arrow). (b) EDS spectrum of total area of a showing C, O, Ca and Au peaks. Elemental EDS map for C (c), O (d), Ca (e) and Au (f). C, O and Ca element peaks are resolved without Au peaks interference. Calcium carbonate nanoparticles (arrows) can be identified in BSE image and elemental maps.

clear distinction between the carbon signal from calcium carbonate and from the adhesive tape (Fig. 6c). In addition, the carbon adhesive tape was $250 \mu\text{m}$

thick, presenting shape discontinuities on its surface. It was composed of $\sim 70\%$ of carbon and 30% of oxygen. These elements represented a noise for carbon and oxygen in calcium carbonate identification in the EDS distribution maps (Fig. 6c and d).

There was no difference between Jaguaribe River estuarine samples directly disposed on the Au stubs and when they were adhered with LSB glue. However, EDS element distribution maps of bulk particles and aggregates directly disposed on the Au stubs were not easy to perform due to charge up,

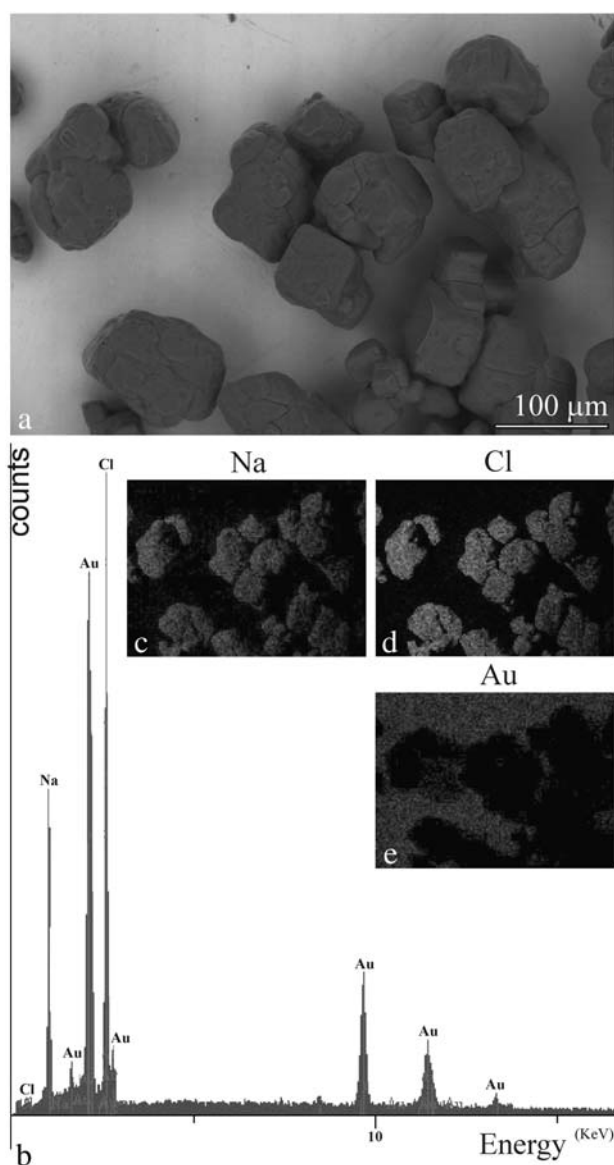


Fig. 5. (a) NaCl disposed on Au stub (BSE image). (b) EDS spectrum of total area of a shows Na, Cl and Au peaks. Elemental EDS maps for Na (c), Cl (d), and Au (e). Na and Cl element peaks are resolved without Au peaks interference.

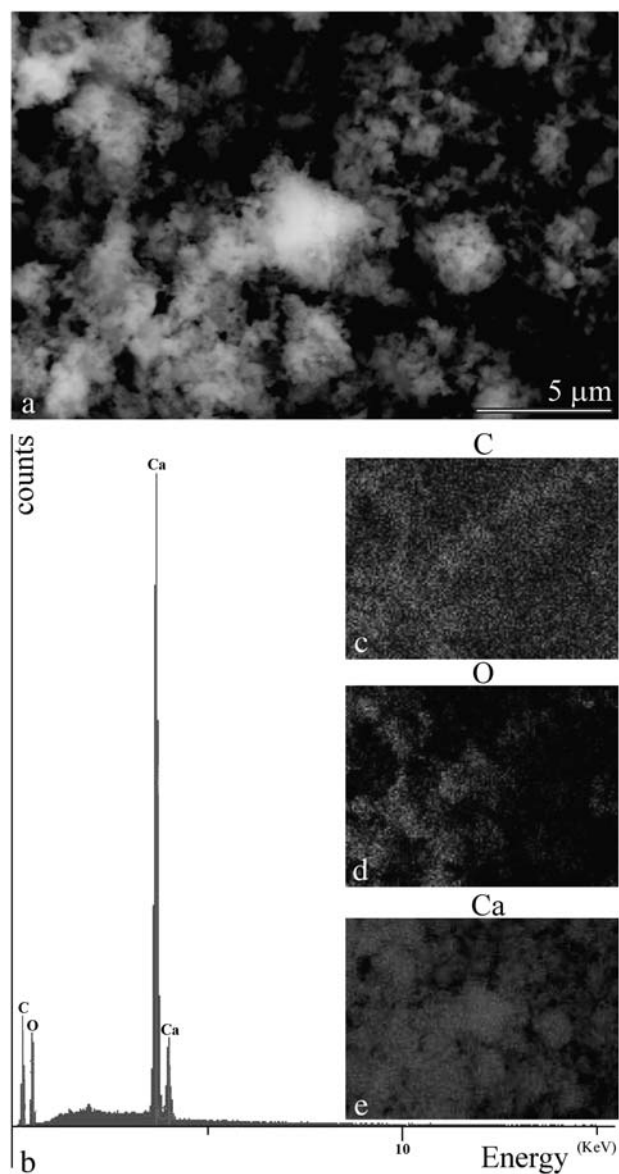


Fig. 6. (a) CaCO_3 adhered onto carbon adhesive tape (BSE image). (b) EDS spectrum of total area (in a) shows C, O and Ca peaks. Elemental EDS maps for C (c), O (d) and Ca (e). X-ray signal for carbon is homogeneously distributed.

heating and vibration. Consequently, they often skipped off the scanning area during analyses.

Sediment of the Jaguaribe River estuary consisted of particles and aggregates with a diverse morphology that covers a wide range of particle or aggregate sizes (Fig. 7). The morphology and dimensions were similar for all depths in the core. In samples directly disposed on the Au stubs, BSE topographic images (Fig. 8a) revealed details of the relief of sediment particles and aggregates better than SE images (Fig. 8b). The edge effect of the SE images was a

function of structure dimensions and accelerating voltage (25 kV).

SEM/EDS analysis of samples directly disposed on the Au stubs showed that the bulk sediment usually had small particles adhered onto their surface (Fig. 9a). The contents of oxygen (O), aluminium (Al), silicon (Si) and metals like magnesium (Mg), potassium (K) and iron (Fe) were frequently revealed in EDS spectra (Fig. 9b), indicating the presence of clay minerals. Silica was also often identified. Calcium was the trace element in a few numbers of sediment bulk particles. The overlapping of O (Fig. 9c), Mg (Fig. 9d), Al (Fig. 9e), Si (Fig. 9f), K (Fig. 9g) and Fe (Fig. 9h) in the EDS element distribution maps was easily identified, suggesting ferromagnesian feldspars.

High magnification of calcium carbonate nanoparticles (<1 μm) directly disposed on the Au stubs presented an irregular aspect in SE (Fig. 10a) or in BSE images. Carbon, oxygen, calcium and gold were detected in EDS spectra (Fig. 10b). The overlapping of carbon (Fig. 10c), oxygen (Fig. 10d) and calcium (Fig. 10e) in the EDS element distribution maps was easily recognized. Gold signal in EDS element distribution maps was from the Au stub (Fig. 10f). Occasionally, gold signal could be detected when flat nanoparticles were analysed.

Calcium carbonate from biological debris was observed in all depths in the core (Fig. 11a). EDS spectra indicated a diversity of chemical elements in these fragments (Fig. 11b). EDS distribution maps in-

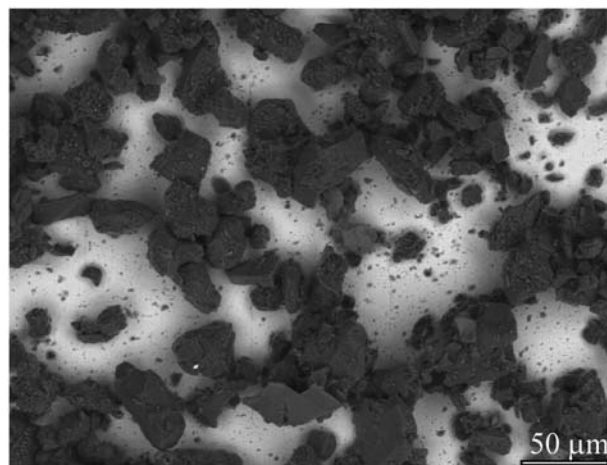


Fig. 7. Sediment of the Jaguaribe River estuary (5–10 cm depth) directly disposed on Au stub observed at low magnification (BSE image). Note diversity of morphology and size of the particles and aggregates.

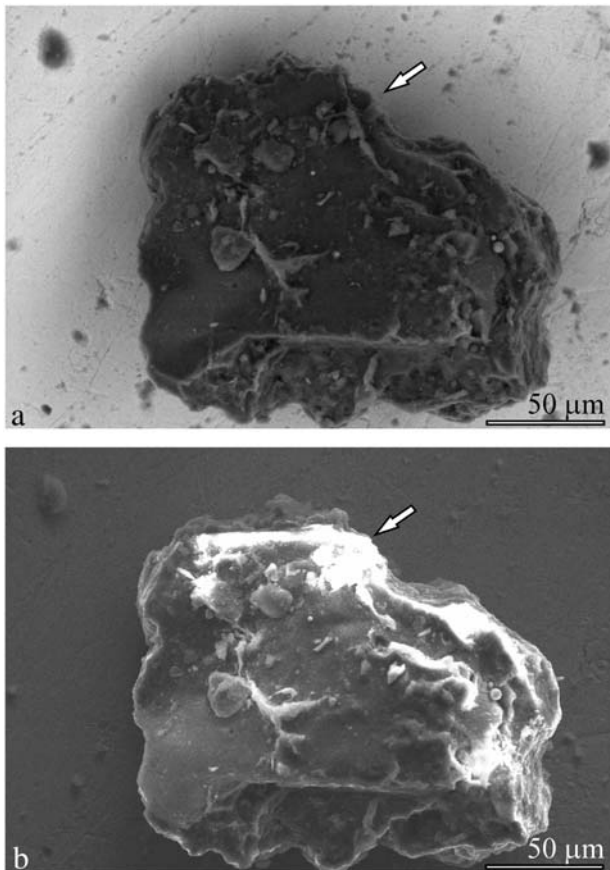


Fig. 8. Particle from Jaguaribe River estuarine sediment (65–70 cm depth) directly disposed on Au stub. (a) BSE image shows actual relief of the sample (arrow), while (b) SE image presents strong edge effect (arrow).

dedicated carbon (Fig. 11c), oxygen (Fig. 11d) and calcium (Fig. 11e) as major elements. Aluminium (Fig. 11f), silicon (Fig. 11g), magnesium (Fig. 11h) and potassium (Fig. 11i) were minor elements. Sodium (Fig. 11j) and chloride (Fig. 11k) presented identical allocation, whereas iron (Fig. 11l) and sulphur (Fig. 11m) were trace elements. However, crystals and framboids (Fig. 11n) of pyrite could be detected at high magnification. The overlap of these elements (Fig. 11o and p) was observed in the EDS distribution maps.

Discussion

SEM/EDS has been proposed as an ideal tool in mineralogy. However, several features can prevent the acquisition of useful EDS spectra as well as several ways in which EDS can be misinterpreted. Mineral specimens are conventionally mounted on aluminium stubs with conductive paint or carbon tape

and coated with a conductive coating such as carbon layer. However, X-ray production from carbon tape and carbon coating, as well as from the aluminium stub itself, prevents the accurate detection of carbon and aluminium in EDS mainly in small specimens

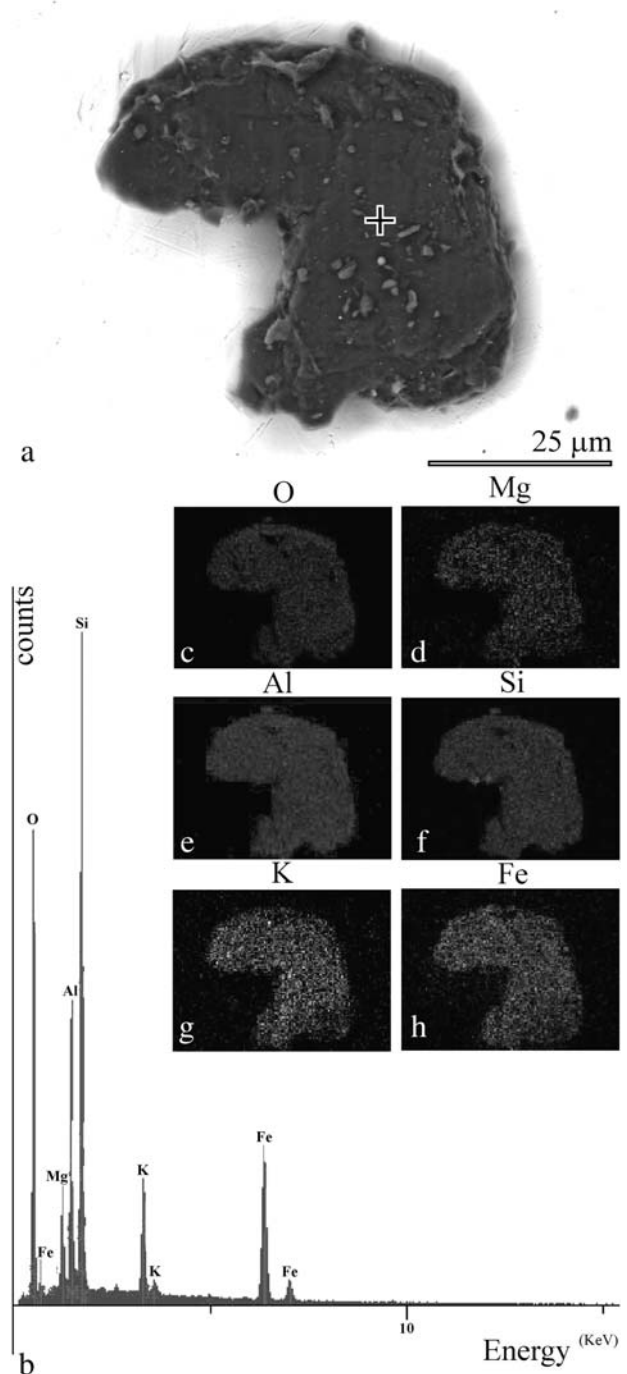


Fig. 9. (a) Clay bulk particle from Jaguaribe River estuarine sediment (5–10 cm depth) directly disposed on Au stub. (b) EDS spectrum (plus sign denotes the analysis point) shows peaks of O, Mg, Al, Si, K and Fe. Note that Au peaks were not detected in point X-ray microanalysis. Elemental map for O (c), Mg (d), Al (e), Si (f), K (g) and Fe (h). Overlapping of element maps is clear.

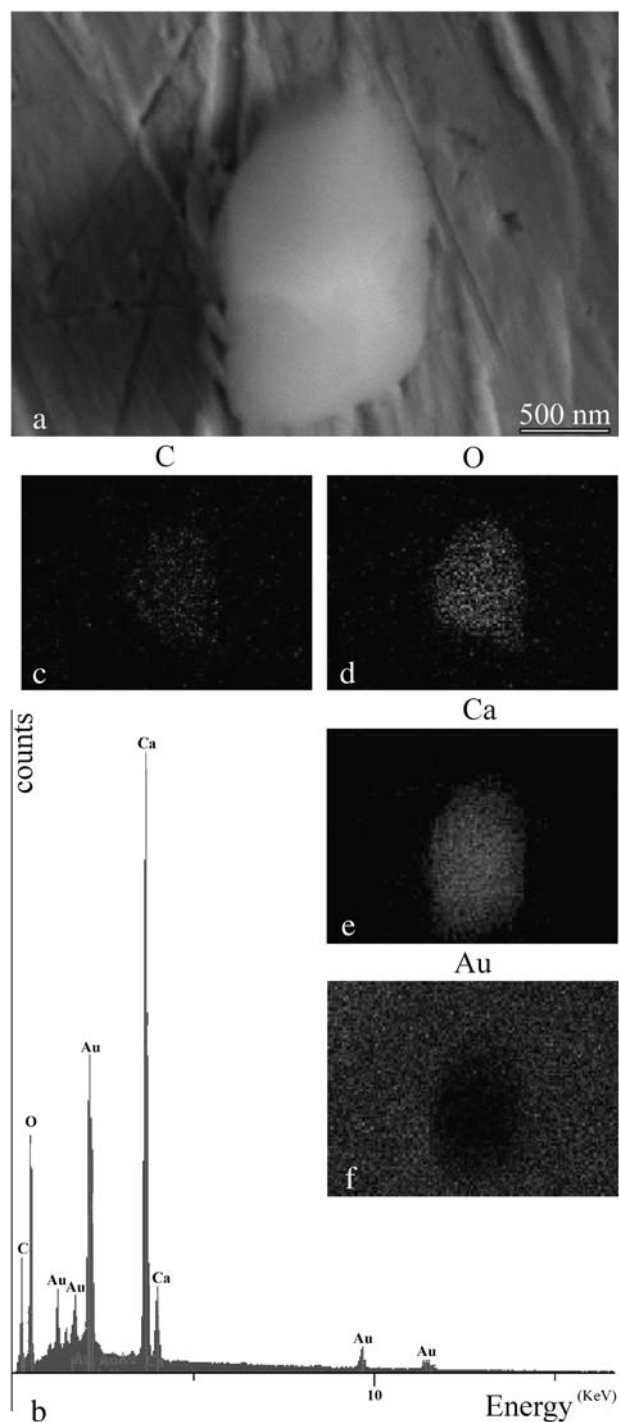


Fig. 10. (a) Calcium carbonate nanoparticle from Jaguaribe River estuarine sediment (65–70 cm depth) directly disposed on Au stub (SE image). (b) EDS spectrum shows peaks of C, O, Ca and Au. Elemental map for C (c), O (d), Ca (e) and Au (f). Note coincident distribution of C, O and Ca. X-ray Au signal was not detected in particle area.

[10]. In Jaguaribe River sediment SEM/EDS description, for example, an accurate detection of clay minerals [19] and calcium carbonate [17] is critical, and the usual protocols based on aluminium stub

and carbon coating could not provide a proper mineralogical interpretation of sediment particles, in particular when involving light elements. The proposed new protocol described in this study apparently solves these challenges as well as minimizes adverse effects of electron beam/sample interaction.

SEM/EDS detection of chemical elements lighter than sodium by conventional procedures is unfeasible when an X-ray detector with a Be window is used [7,11–13]. Spurious signal from carbon tape and coat as well as aluminium from the stub itself masks key signals from calcium carbonate and clay minerals from samples [16]. SEM/EDS analysis of controls showed that gold support and thin film of adhesive glue do not produce deleterious signals for calcium carbonate and clay minerals. In Au stub covered with a thin film of LSB glue, the carbon peak was detected but close to background level and undetectable in its elemental map. In contrast, bulk and nanoparticles calcium carbonate adhered onto Au stub with LSB glue showed clear carbon peak profiles and carbon signal from LSB glue was undetectable in their elemental distribution maps. In addition, counterproof SEM/EDS analysis confirms previous records [10] in which carbon tape and coating as well as aluminium stub proved inappropriate to several mineral characterizations.

In the range of available choices, Au was elected because it is an uncommon element in the environment. Clay minerals are predominant in estuarine sediments. They have aluminium and silicon as major elements. In addition, pyrite—a common mineral in this environment—has iron and sulphur. So, Au can prevent EDS spectra misinterpretation. Unfortunately, P and Au are non-distinguishable by EDS. In general, this method is inappropriate to detect P. However, P could be detected when large-particle point spectrum were carried out on sediment particles and aggregates.

Radiation damage can adversely affect samples during SEM examination. Electron beam heating is due to the incoming electrons and sample interaction. In the case of SEM, a bulk specimen is normally used and heat flow is radial in three dimensions, leading to a relatively small temperature rise [14]. Shaking of the samples has a deleterious heating effect when sediment bulk specimens disposed on gold mounting discs were submitted to long-term SEM/EDS analysis.

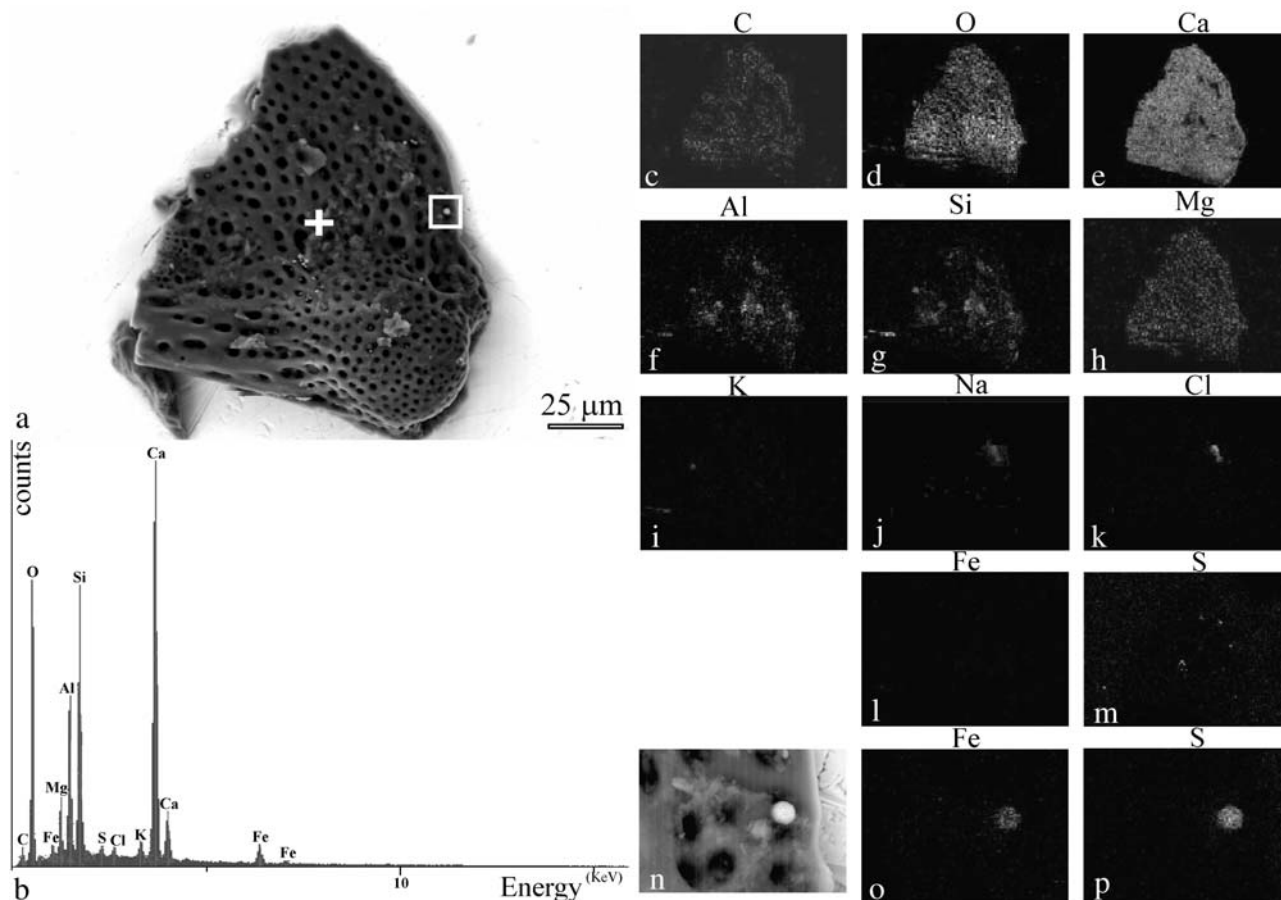


Fig. 11. (a) Biological fragment from Jaguaribe River estuarine sediment (65–70 cm depth) directly disposed on Au stub (BSE image). (b) EDS (plus sign denotes analysis point) spectrum shows peaks of C, O, Na, Mg, Al, Si, S, Cl, K, Ca and Fe. Elemental EDS map for C (c) O (d), Ca (e), Al (f), Si (g), Mg (h), K (i), Na (j), Cl (k), Fe (l) and S (m). High magnification of the particle (squared area in a) revealed pyrite framboid (n). Elemental EDS map for Fe (o) and S (p) indicate overlap of these elements.

Usually, large mineral specimens are mounted on stubs with conductive paint or carbon tape to avoid this effect [10].

According to the literature [12,13], the most efficient production of X-rays generally occurs when the bombarding electrons have about three times the X-ray energy and, therefore, there should be no difficulty in analysing all the elements for a SEM operating at 25–30 kV. Chemical elements in Jaguaribe estuarine sediments are not wholly identified, mainly minor and trace elements. On the other hand, analyses of all elements are essential in environmental research.

Geological samples are commonly non-conducting and the possibility that a buildup of negative charge in the region penetrated by incident electrons must be considered [7]. Bulk sediment particle shaking was a consequence of the charging up when samples were disposed on Au stubs. LSB glue was used to

prevent shaking of sediment particles. In contrast, sediment particles <1 µm were easily analysed when directly disposed on Au stub.

Sediment bulk sample shaking was minimized by controlled low temperature of the specimen holder and glue. Electrostatic charging can also affect samples during examination in a SEM which is solved by coating the surface of the specimen with a thin film of metal or conducting carbon [7,10–13]. Our strategy to reduce charging damage used a gold stub for Jaguaribe River sediments SEM/EDS analysis.

Spatial and concentration elements resolution is a function of the atomic number, element concentration and grain size [7,10–13]. In our Jaguaribe River sediments examination, EDS spectra revealed the diversity of chemical elements, while EDS distribution maps indicated its probable stoichiometry.

In the Jaguaribe River estuarine sediments, the morphology of the particles typically varies from bio-

logical to non-biological origin. Particle rates also vary according to sediment depth, probably reflecting changes in regional climate and hydrology [18]. Silica minerals, aluminosilicates, sodium chloride and pyrite have been described as predominant compounds in the non-biological portion [16]. However, it is an environment where carbonate deposition plays a primary role in regional carbon cycle [17] as well as carbon is obviously a key element of the carbonate global system [1,2,15]. In this study, we could obtain important results to clarify calcium carbonate characterization in sediments by combination of a new protocol and EDS experiments. In addition, our results agree with previous reports which suggest ferromagnesian feldspar occurrence, the dominating mineral of most of the river lower basin [19].

Concluding remarks

The new procedure proposed was efficient in identifying carbonates in estuarine sediments and control salts without the noise from sample mounting disc and adhesive paint or carbon tape. In addition, gold mounting base in association with controlled sample base temperature prevents damage from electron beam/specimen interaction without carbon coating. The disadvantage was vibration and heating when samples were deposited on mounting disc without glue. Good images were very difficult to obtain and demand a lot of time by this way. However, we believe that there exists a positive balance between accurate information versus sample damage when this new protocol was applied to the Jaguaribe River estuarine sediments.

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