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A regenerable ruthenium tetraammine nitrosyl complex immobilized on a modified silica gel surface: Preparation and studies of nitric oxide release and nitrite-to-NO conversion

Fabio Gorzoni Doro [a], Ubirajara P. Rodrigues-Filho [b], E. Tfouni [a,*]

[a] *Departamento de Química, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Av. dos Bandeirantes 3900, 14040-901 Ribeirão Preto, SP, Brazil*
[b] *Instituto de Química de São Carlos, Universidade de São Paulo, CP 780, 13560-970 São Carlos, SP, Brazil*

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Abstract

Silica gel bearing isonicotinamide groups was prepared by further modification of 3-aminopropyl-functionalized silica by a reaction with isonicotinic acid and 1,3-dicyclohexylcarbodiimide to yield 3-isonicotinamidepropyl-functionalized silica gel (ISNPS). This support was characterized by means of infrared spectroscopy, elemental analysis, and specific surface area. The ISNPS was used to immobilize the $[Ru(NH_3)_4SO_3]$ moiety by reaction with *trans*-$[Ru(NH_3)_4(SO_2)Cl]Cl$, yielding $[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(SO_3)]$. The related immobilized $[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(L)]^{3+/2+}$ (L = SO_2, SO_4^{2-}, OH_2, and NO) complexes were prepared and characterized by means of UV–vis and IR spectroscopy, as well as by cyclic voltammetry. Syntheses of the nitrosyl complex were performed by reaction of the immobilized ruthenium ammine $[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(OH_2)]^{2+}$ with nitrite in acid or neutral (pH 7.4) solution. The similar results obtained in both ways indicate that the aqua complex was able to convert nitrite into coordinated nitrosyl. The reactivity of $[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(NO)]^{3+}$ was investigated in order to evaluate the nitric oxide (NO) release. It was found that, upon light irradiation or chemical reduction, the immobilized nitrosyl complex was able to release NO, generating the corresponding Ru(III) or Ru(II) aqua complexes, respectively. The NO material could be regenerated from these NO-depleted materials obtained photochemically or by reduction. Regeneration was done by reaction with nitrite in aqueous solution (pH 7.4). Reduction–regeneration cycles were performed up to three times with no significant leaching of the ruthenium complex.

Keywords: Nitric oxide; Nitrite; Conversion; Silica gel; Ruthenium; Nitrosyl; Controlled; Photochemistry; Tetraammine; Aminopropylsilica; Functionalized silica; Nitric oxide donor

1. Introduction

Nitric oxide (NO) is an important biomolecule that plays a pivotal role in several physiological processes such as blood pressure regulation, inhibition of smooth muscle proliferation, and platelet aggregation [1]. The discovery of the roles NO plays in biological processes launched a spectacular increase in investigation on the subject. The physiological functions of NO are complicated and depend on thermodynamic, kinetic, and concentration considerations [2,3]. High or low concentrations of NO can be either beneficial or harmful, depending on the circumstances [1–4]. Thus, there is a continuing need for controlled and site-specific NO delivery agents. Several NO donors and scavengers, such as metal complexes, have been reported [5–31]. Immobilization of NO donors on solid supports aiming at potential biological application has received attention in the past few years and many very interesting papers have been published [32–46].

Among a series of important supports, silica is widely used as a supporting material since it presents desirable properties such as mechanical and thermal resistance. In addition to these properties, the surface of silica can be chemically modified by attaching a great variety of pendant functional groups due to the presence of silanol and siloxane groups in the in-

* Corresponding author. Fax: +55 16 3602 4838.
E-mail address: eltfouni@usp.br (E. Tfouni).

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organic framework [47]. This strategy provides access to quite useful materials [48]. Covalent immobilization onto solid support of a chelate moiety enables interaction with metal ions or metal complexes [49–53]. In an earlier work, Franco and co-workers [53] explored this property to prepare a series of immobilized ruthenium ammine complexes on a silica surface bearing 3-(imidazolyl)propyl groups. They also verified that the immobilized complexes show behavior similar to those in solution.

As stated above, a new class of materials that have attracted attention in recent years is formed by nitric-oxide-releasing materials [33,35,37–41,54]. A wide range of moieties such as diazeniumdiolates [55], nitrosothiols [56], and metal nitrosyls [37,39–41,44] have been immobilized onto the surface or into the backbone of organic or inorganic polymeric matrices such as silicone rubbers, polyurethane, poly(vinyl chloride), poly(vinyl alcohol), poly(methyl methacrylate), silica, and organoxerogels [33,34,38–40,45,54,56–60]. Among them, the immobilization of metal nitrosyls is of particular interest, because it offers several possibilities for NO release by designing the complexes as well as the support characteristics [37,39–41,61].

Recently it was found that immobilization of cobalt or ruthenium complexes with salen ligands into an organic polymeric backbone leads to materials that are able to take up or release nitric oxide [37,57]. Ruthenium nitrosyl complexes with cyclam (cyclam = 1,4,8,11-tetraazacyclotetradecane) [39] and salen (N,N'-bis-(salicylidene)ethylenediaminato) ligands [40] immobilized into xerogel matrices are able to release nitric oxide upon light irradiation. Regeneration of the former was made by reaction with NO [39] and that of the latter with nitrite solution after NO photolabilization [40].

The materials mentioned involve the immobilization of the complex by entrapment in solid inorganic silica xerogels. On one hand, this strategy offers the advantages of preserving the complex, avoiding or minimizing leaching, and possibly providing additional control of NO release. On the other hand, it somewhat narrows the access of reducing agents that are known to promote NO release by reduction of the coordinated nitrosyl group [8] as well as the NO diffusion from the bulk to the surface of the material. Especially for an NO donor strategy based on the chemical reduction of the coordinated NO by biochemical reducing agents present in body fluids, the access to more deeply entrapped Ru–NO species may be delayed or even prevented; in fact, this may become an additional reactivity control.

Very recently, Franco and co-workers [61] reported the immobilization of the [Ru(NO)(edta)] by amide bonding formation onto a silica surface modified with [3-(2-aminoethyl)aminopropyl)triethoxysilane groups (AEATS), denoted as AEATS/$Ru^{II}NO^{+}$. The authors did not observe significant differences between the immobilized complex and the analogous in solution. Noteworthily, they also found that the AEATS/$Ru^{II}OH_2$ was able to convert NO_2^- to coordinated NO at pH 7. Although a catalytic cycle has been proposed for this reaction, no further data were provided regarding the stability of the immobilized complex toward reduction–regeneration cycles, and NO photolabilization was not reported.

As a part of our interest in the chemistry of ruthenium nitrosyl complexes, we have decided to explore the immobilization of the Ru–NO moiety on a silica surface. Surface immobilization could promote easier access for reducing agents, leading to NO release and possibly leading to more stable materials from a recycling point of view. In this way, the immobilization of the Ru(II) complexes *trans*-$[Ru(NH_3)_4L(NO)]^{3+}$ (L = N heterocyclic ligands) seems to be a natural choice. These complexes, which can be formally described as Ru^{II}–NO^{+} complexes [8,62], have been studied [8], their chemical and photochemical properties are known [8,63], and they have shown biological activity and low toxicity [64–66]. They are also able to release NO photochemically, with an irradiation wavelength dependence, or via reduction [8,18,63]. Immobilization of ruthenium ammines can provide some advantages over other complexes. The properties that lead to the release of NO, for instance, reduction potentials of the complexes, spectra and, thus, excited states, and NO rate of release (fast or slow), can be tuned by judicious choice of the L ligand and can be triggered by an adequate choice of reducing species and/or irradiation wavelength. Considering that Ru-based materials already showed that immobilization does not change the complexes' reactivity [39,53,61], the properties observed for *trans*-$[Ru(NH_3)_4L(NO)]^{3+}$ complexes could be extended to the related ruthenium-based materials. One possible strategy for preparing such materials could be the attachment of heterocyclic bases to the silica surface. In fact, silica gels bearing heterocyclic bases such as pyridine [67], 2-aminoethylpyridine [52], and imidazol [49] are already known.

In solution, upon chemical or electrochemical reduction, the *trans*-$[Ru(NO)(NH_3)_4(L)]^{n+}$ (L = N heterocyclic ligands)-like complexes release nitric oxide, leading to the respective aqua ruthenium(II) complex, *trans*-$[Ru(OH_2)(NH_3)_4(L)]^{(n+1)+}$ [8]. Irradiation of ruthenium ammine nitrosyl complexes in aqueous solution with photons of energies corresponding to the bands in the range 300–350 nm results in nitric oxide aquation and formation of the corresponding aqua ruthenium(III) complex [63]. Both Ru(II) and Ru(III) ammine complexes, *trans*-$[Ru(OH_2)(NH_3)_4(L)]^{n+}$, are robust and thermodynamically stable, and except for the aqua ligand in the Ru(II) complex, all other groups are kinetically inert with respect to substitution [68,69]. The corresponding nitrosyl complexes are also robust and thermodynamically stable, and all ligands are inert with regard to substitution reactions [8].

Aiming to contribute to a better understanding of the properties of immobilized NO donors, we report on the preparation and characterization of immobilized ruthenium ammine complexes on silica surfaces bearing isonicotinamide groups, $[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(L)]^{3+/2+}$ (L = SO_2, SO_4^{2-}, OH_2, and NO). The choice of the peptide bond to link the pyridine derivative group was made based on widespread use of matrices bearing amino groups [48,70]. These matrices can be further modified with coordinating groups that can bind to a ruthenium moiety. Special attention was devoted to $[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(NO)]^{3+}$ to evaluate the release

of NO by chemical reduction and upon light irradiation. The chemically or photochemically NO-depleted material was investigated regarding its regeneration potential by reaction with nitrite. Reduction–regeneration cycles were studied in order to evaluate the chemical stability of the immobilized complex, as required for biomedical applications.

2. Experimental

2.1. Chemicals and reagents

Isonicotinic acid (Aldrich) was recrystallized from hot water, dried under vacuum, and further dried overnight at 80 °C previous to use. 3-Aminopropyl-modified silica gel (APS) (Aldrich) (∼1 mmol NH_2 g^{-1}, particle size 40–63 μm) was dried overnight at 110 °C. 1,3-Dicyclohexylcarbodiimide (DCCI) (Aldrich) was distilled under reduced pressure (∼10^{-4} Torr). Dimethylformamide (DMF) (Aldrich) was dried over molecular sieves and distilled under reduced pressure right before use. $RuCl_3 \cdot nH_2O$ (35–40% Ru, Acros) was used as a ruthenium source for the synthesis of complexes. $[RuCl(NH_3)_5]Cl_2$, *trans*-$[Ru(NH_3)_4(HSO_3)_2]$, *trans*-$[Ru(NH_3)_4(SO_2)Cl]Cl$, and *trans*-$[Ru(NO)(NH_3)_4(isn)](BF_4)_3$ were synthesized as previously described in the literature [71–73]. Other reagents and solvents were of analytical grade and used as supplied. All the manipulations with air-sensitive compounds were carried out following conventional techniques [74].

2.2. Electrochemical measurements

Cyclic voltammetry experiments were performed using a Princeton Applied Research (PAR) Model 273 potentiostat/galvanostat. The three-electrode system used in these studies consisted of a working electrode (modified carbon paste electrode), an Ag/AgCl reference electrode, and a platinum wire as counter electrode. Modified carbon paste electrodes were prepared by mixing a desired amount of immobilized complexes (20%) with carbon paste (75%) and nujol oil (5%). All measurements were carried out at 25 °C.

2.3. Electronic and vibrational spectra

Electronic spectra were recorded using a Hewlett-Packard 8452A spectrophotometer. Since the refraction indices of carbon tetrachloride and silica matrix are nearly the same [75], the supported complexes on silica gel surface were immersed in spectra grade carbon tetrachloride and the spectrum of the suspension was obtained using a quartz cell of path length 1 mm. Infrared (IR) spectra were obtained in a Bomem MB 102 FTIR spectrophotometer using KBr pellets in the range 4000–400 cm^{-1}.

2.4. Photolysis experiments

Monochromatic irradiations at 334 nm were carried out using a 150-W Xenon lamp in a Model 6253 Oriel Universal Arc Source. Irradiations with white light were performed using an IR filter light beam in the same system. The progress of the NO photolabilization was monitored by infrared spectroscopy. Photolysis experiments with the immobilized ruthenium nitrosyl complex were performed in a pellet prepared by dispersing the material in KBr. This same pellet was then irradiated with light and IR spectra were recorded during time intervals.

2.5. NO measurements

NO release was measured with a NO meter from Innovative Instruments Inc. This apparatus detects NO concentration directly by an amperometric technique. The calibration curve was constructed by addition of aliquots of a nitric oxide saturated stock solution. This solution was prepared by degassing aqueous phosphate buffer solution (pH 7.4) followed by introduction of nitric oxide as previously described [4]. NO was generated from addition of HNO_3 solution (ca. 33%) to copper wires [76] and passed by a 10 M KOH solution to remove NO_x species. The NO concentration was calculated according to the reported molar fraction solubility of NO (2.1 mM at 25 °C) which was confirmed by titration with $KMnO_4$ as previously described [77].

For the NO measurement from chemical reduction experiments, an amount of the nitrosyl-immobilized complex, $[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(NO)]^{3+}$, was suspended in 10 ml of previously degassed phosphate buffer (pH 7.4) in a 25-ml rounded vessel and an excess of Eu(II) (30 times) was added. A stream of argon was kept over the solution during the measurement to minimize oxygen presence.

2.6. Ruthenium analysis

Determinations of the amount of immobilized complexes on silica were performed as follows: the immobilized ruthenium complex (20–30 mg) was treated with KOH 2 M (4 ml) solution, NaOCl 5% (1 ml), and 20 mg of potassium persulfate and the mixture was heated almost to the boiling point. After cooling to room temperature, the volume was adjusted to ca. 10 ml. The ruthenium concentration was determined by absorbance measurement at 415 nm, according to the method described by Malouf [78].

2.7. Specific area determination

The determination of the surface area of the silica samples by the BET [79] method was done on Quanta Chrome Model NOVA 1200 equipment.

2.8. Preparation of silica containing isonicotinamide groups

Silica bearing isonicotinamide groups was prepared by a selective amidation reaction of amino groups of APS using 1,3-dicyclohexylcarbodiimide [80] and isonicotinic acid. Isonicotinic acid (1.5 g, 7.5 mmol) was dissolved in 100 ml of dry DMF previously heated to 60 °C and 1,3-dicyclohexylcarbodiimide (2.5 g, 7.5 mmol) was added. After a few minutes, 2.5 g of APS (2.8 mmol NH_2 g^{-1}) was suspended in the mixture. The

suspension was mechanically stirred and kept under dry nitrogen for 4 h. The resulting solid was filtered, washed with DMF, water:methanol (1:1), and methanol, and extracted in a Soxhlet apparatus overnight with methanol in order to remove any excess of reagent and dried in an Abderhalden pistol at 60 °C for 2 h.

2.9. Preparation of nitrosyl ruthenium ammine immobilized on silica gel surface bearing isonicotinamide groups

Immobilization of the ruthenium ammine complex *trans*-$[Ru(NH_3)_4(SO_2)Cl]Cl$ was performed in a way similar to that described previously [53] and the synthesis of the nitrosyl-immobilized $[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(NO)]^{3+}$ complex follows a procedure similar to those described for the complex in solution [73]. The basic procedure is as follows.

$[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(SO_3)]$. A quantity of 50 mg ($1.6 \times 10^{-4}$ mol) of *trans*-$[Ru(NH_3)_4(SO_2)Cl]Cl$ was added to 10 ml of previously degassed $NaHCO_3$ 0.4 mol L^{-1} aqueous solution and 0.25 g of ISNPS was then added. The suspension was kept under continuous argon bubbling. The deep orange solid was collected by filtration. This immobilized complex is easily oxidized by air.

$[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(SO_2)]Cl_2$. A quantity of 0.25 g of the immobilized ruthenium ammine sulfito complex, $[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(SO_3)]$, freshly prepared, was washed with 50 ml of 0.1 mol L^{-1} HCl degassed solution, ethanol-dried, and then stored under vacuum.

$[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(SO_4)]Cl$. A quantity of 0.25 g of the immobilized ruthenium ammine sulfur dioxide complex, $[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(SO_2)]Cl_2$, was suspended in 10 ml of 0.1 mol L^{-1} HCl solution and oxidized by addition of 1 ml of H_2O_2 (3%) solution. The pale yellow solid was separated from solution by filtration, washed with ethanol, dried,and stored under vacuum.

$[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(OH_2)]Cl_2$. A quantity of 0.25 g of the immobilized ruthenium ammine sulfate complex, $[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(SO_4)]Cl$, was suspended in 10 ml of a previously degassed 0.1 mol L^{-1} HCl solution and 1.5 ml of 0.05 mol L^{-1} Eu(II) solution was added. The pale yellow suspension readily turned to a deep red color. The immobilized complex was separated from solution by filtration in a glove bag and washed with 30 ml of degassed 0.1 mol L^{-1} HCl solution and immediately used in the next step.

$[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(NO)]^{3+}$. Method 1: A quantity of 0.25 g $[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(OH_2)]Cl_2$ was suspended in 10 ml of 0.1 mol L^{-1} HPF_6 aqueous solution, previously degassed, and an excess of $NaNO_2$ (100 mg) was added. The red suspension turned to yellow. The solid was collected by filtration, washed with deionized water and ethanol, dried, and then stored under vacuum. *Method 2*: Alternatively, the $[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(NO)]^{3+}$ was prepared by suspending the immobilized aqua complex in 10 ml phosphate buffer solution (pH 7.4) containing 1 mol L^{-1} of $NaNO_2$. The red suspension turned slowly to yellow. The solid was separated from solution by filtration, washed with phosphate buffer, deionized water, and ethanol, dried, and stored under vacuum.

2.10. Recycling experiments

Recycling experiments were performed in two ways. One consists of reacting the immobilized nitrosyl complex with Eu(II) ions in a way similar to that described for the preparation of $[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(OH_2)]Cl_2$. Regeneration was achieved by reaction of the aqua species with $NaNO_2$ solution as described for the preparation of $[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(NO)]^{3+}$ (*method 2*). Another method was performed after photolysis of $[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(NO)]^{3+}$ in a KBr pellet. The photolyzed KBr pellet was dissolved with 2 mol L^{-1} $NaNO_2$ aqueous solution (2 ml) and the solid was recovered by filtration, washed with deionized water and ethanol, and dried under vacuum.

3. Results and discussion

3.1. Preparation and characterization of silica gel bearing isonicotinamide groups

The attachment of isonicotinic acid onto 3-aminopropyl silica gel was performed in a one-step reaction by selective amidation of free amino groups of APS using DCCI [80] yielding silica bearing isonicotinamide groups, named ISNPS, as presented in Scheme 1.

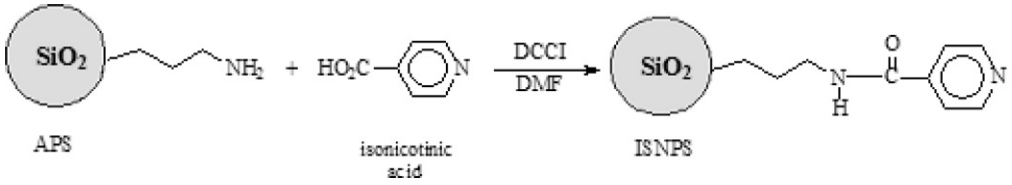
The number of functional amino and isonicotinamide groups attached to the silica surface was determined by considering the elemental analysis data. The specific surface area S_{BET} was also determined allowing an estimate of the density of functional groups, d, and the average intermolecular distance, l, by assuming a uniform distribution on the matrix surface [81],

$$d = (N \times N_A)/S_{BET}, \quad (1)$$

$$l = (1/d)^{1/2}, \quad (2)$$

where N is the number of moles of the functional groups per gram of silica and N_A is Avogadro's number. These data are summarized in Table 1.

As shown in Table 1, an amount of 0.66 mmol of isonicotinamide per gram of support was found in ISNPS, corresponding to approximately 58% conversion of the amino groups to



Scheme 1. Preparation of isonicotinamide-functionalized silica gel.

Table 1
Elemental analysis data, surface area, number of moles (N), surface density (d), and mean interatomic distance (l) of functional groups

Silica	Elemental analysis		S_{BET} ($m^2\ g^{-1}$)	N ($mmol\ g^{-1}$)	d ($group\ nm^{-2}$)	l (Å)
	% N	% C				
APS	1.59 ± 0.03	4.97 ± 0.09	332 ± 5	1.13 ± 0.08[a]	2.1 ± 0.2	6.9 ± 0.5
ISNPS	2.59 ± 0.02	9.98 ± 0.12	304 ± 5	0.66 ± 0.05[b]	1.3 ± 0.1	8.7 ± 0.6

[a] NH_2.
[b] Isonicotinamide.

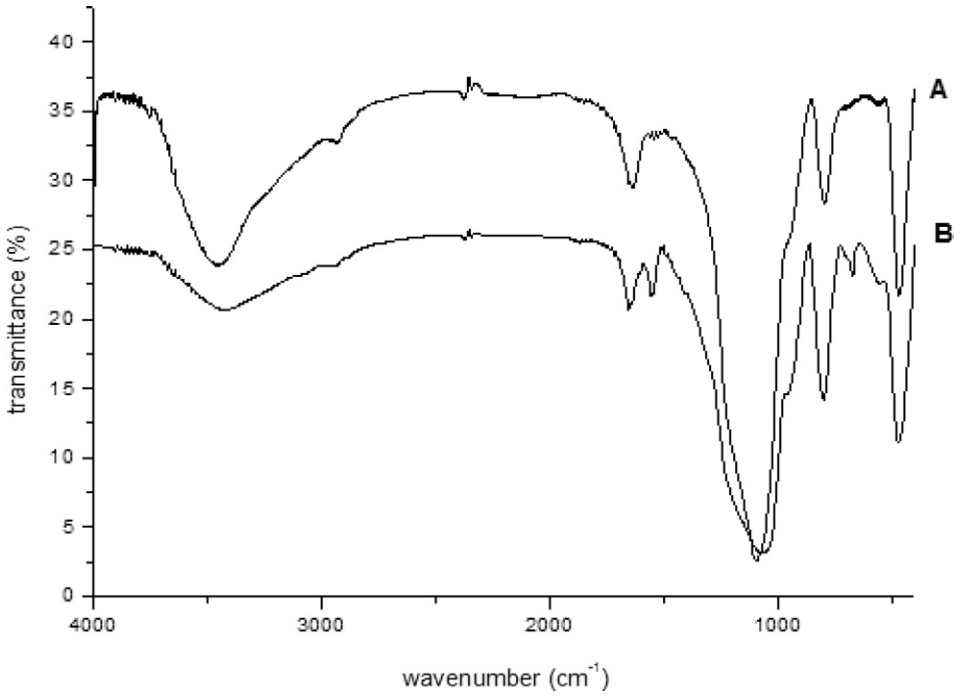


Fig. 1. Infrared spectra of APS (A) and ISNPS (B) in KBr pellet.

amide groups. This percentage of conversion is near to the physical limit because isonicotinamide groups possibly do not bind orthogonally to the surface but in an angular shape occupying a volume larger than the isonicotinamide ring. These values are in agreement with those reported by Jaroniec and co-workers [82] for the preparation of a series of amide bound phases on silica gel surface. Therefore, the resulting modified surface is a mixture of amine and isonicotinamide functional groups. The specific surface area determined by gaseous nitrogen adsorption [79] showed a decrease from $332.8 \pm 5\ m^2\ g^{-1}$ for APS to $304.9 \pm 5\ m^2\ g^{-1}$ for ISNPS. This fact has been attributed to the attachment of pendant groups that partially block the adsorption of nitrogen molecules on the silica surface [52].

The immobilization of isonicotinamide groups can be confirmed by infrared spectra for the APS and ISNPS (Fig. 1).

The main features of APS and ISNPS IR spectra are the appearance of bands associated with the inorganic backbone. There is a large broad band around 3400 cm^{-1}, which is attributed to O–H stretching frequencies of silanol groups and the remaining adsorbed water. The strong band at ~1100 cm^{-1} and a band at 800 cm^{-1} are related to the siloxane stretching ν(Si–O–Si). The shoulder at ~900 cm^{-1} can be assigned to the Si–OH stretching frequency. The band around 1650 cm^{-1} is attributed to the angular vibration of adsorbed water molecules as well as to coupled vibration modes of Si–O–Si [52,83]. A weak band near 2950 cm^{-1} is also observed and can be attributed to C–H stretching [84]. However, the ISNPS presented a characteristic and well-defined band at 1554 cm^{-1}, which does not appear in the IR spectra of APS. This band is also observed for isonicotinamide at 1551 cm^{-1} and it is in the range of the amide II band. Amide I band was expected to appear near to 1660 cm^{-1}. This band is probably enveloped by the band at 1650 cm^{-1}. In addition to the evidence mentioned above, the product of the reaction performed under the same conditions between APS and isonicotinic acid but in the absence of DCCI did not show the band at 1554 cm^{-1}. Furthermore, the material prepared in this way was not able to immobilize the ruthenium complex. These are unequivocal evidence of the presence of isonicotinamide groups on the silica surface.

3.2. Preparation and characterization of immobilized ruthenium ammine complexes

The $[Ru(NH_3)_4(SO_3)]$ moiety was immobilized on the silica surface bearing isonicotinamide groups by reacting *trans*-$[Ru(NH_3)_4(SO_2)Cl]Cl$ in $NaHCO_3$ aqueous solution at pH 9. At this pH, *trans*-$[Ru(NH_3)_4(SO_2)Cl]^+$ is converted to *trans*-$[Ru(NH_3)_4(SO_3)Cl]^-$, followed by fast release of the *trans*

chloride ion, leading to *trans*-$[Ru(NH_3)_4(SO_3)(OH_2)]$ [85], allowing the formation of $[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(SO_3)]$. The Ru(II) sulfito complex is then converted into sulfur dioxide complex by lowering the pH and subsequently oxidizing with H_2O_2. Ru(II) is then converted to Ru(III) and the SO_2 ligand is oxidized to sulfate to form $[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(SO_4)]^+$. Then reduction of Ru(III) to Ru(II) results in the sulfato aquation and the immobilized aqua complex, $[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(H_2O)]^{2+}$, is reacted with nitrite (see Scheme 2) to form the immobilized nitrosyl complex $[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(NO)]^{3+}$.

In a previous work, Franco and co-workers [53] reported the immobilization of the same moiety on a silica surface modified with 3-(imidazolyl)propyl groups. The authors found that the properties of the immobilized ruthenium complexes were similar to those of the complexes in solution. Since in our system the immobilized complexes are also separated from the support by three carbon atoms, we would expect similar behavior. We followed the preparation of the aqua complex with slight modification of the method reported in the literature [53], and the following steps are depicted in Scheme 2.

The ruthenium content was determined for each immobilized complex showing minimal leaching during the preparation steps (Table 2).

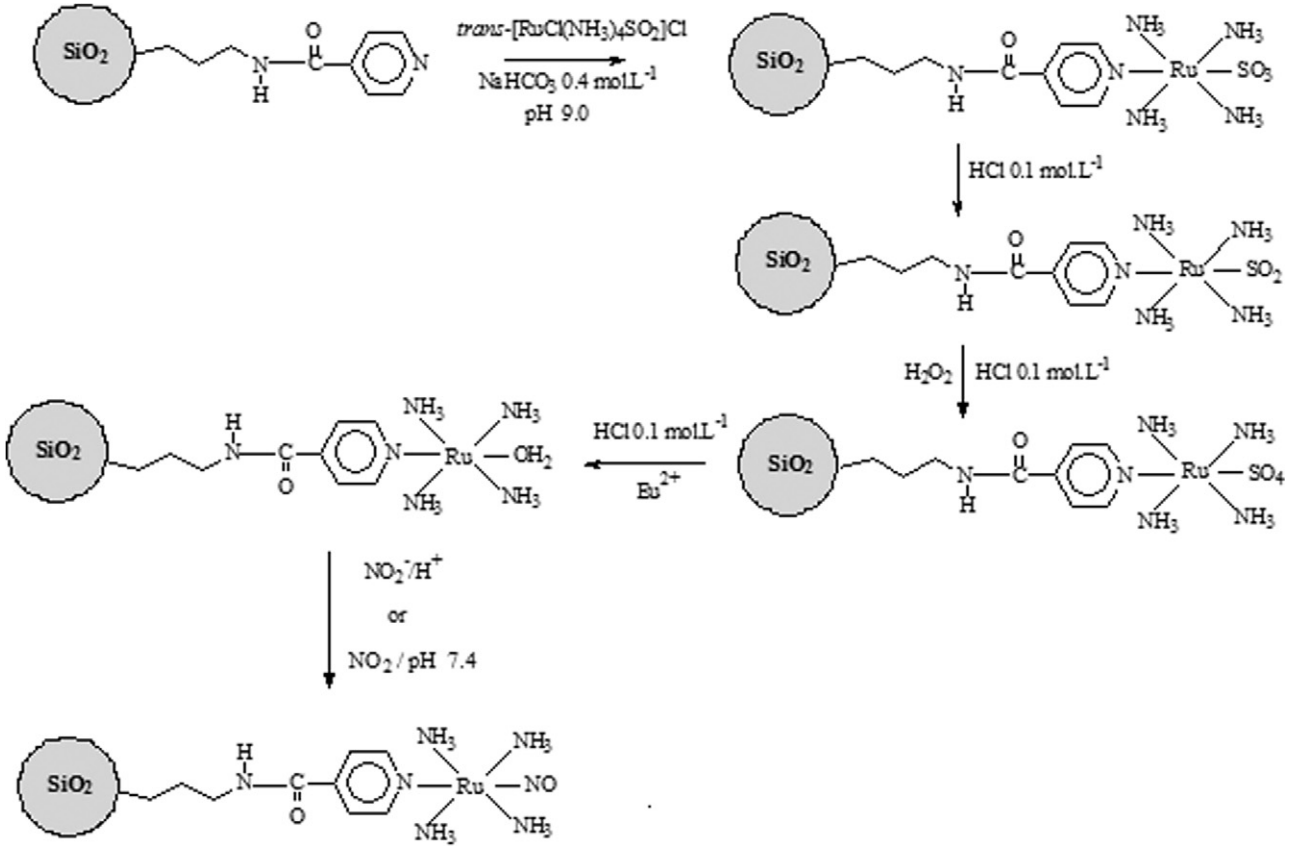
Since the ISNPS surface contains a mixture of isonicotinamide and amino functional groups, immobilization of the *trans*-$[Ru(NH_3)_4(SO_2)Cl]^+$ complex by the NH_2 groups was investigated. Reactions performed with APS under the same conditions as those described for ISNPS resulted only in a minor loading of 7 μmol g^{-1} of ruthenium (less than 3% of 2.8×10^{-4} mol of Ru). This result indicates that the ruthenium complex is attached to the support essentially by the isonicotinamide groups.

As can be seen from Table 2, noticeable leaching occurs only from the first to the second step. This is possibly due to the *trans* labilizing effect of the sulfito ligand [85]. As mentioned earlier, the immobilized Ru(II) and Ru(III) are inert regarding substitution of the ligands, except for the aqua ligand in the Ru(II) species, which, as will be shown latter, would be responsible for the recycling properties of the material. The complex is strongly attached to the support through a peptide bond, a stable bond that is not affected by redox or photochemical processes and prevents its leaching. The amount of ruthenium

Table 2
Loading of complex in the ISNPS support

Supported complex	"Loading" of complex ($\times 10^{-4}$ mol g^{-1} of silica)[a]
$[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(SO_3)]$	2.8
$[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(SO_2)]Cl_2$	2.3
$[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(SO_4)]Cl$	2.1
$[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(OH_2)]Cl_2$	2.1
$[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(NO)](PF_6)_3$	1.9
$[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(NO)](PO_4)_3$	2.0

[a] Based on Ru elemental analysis according to Ref. [78]. Uncertainty is $\pm 0.2 \times 10^{-4}$ mol g^{-1}.



Scheme 2. Representation of the synthesis of the nitrosyl ruthenium ammine-immobilized species.

complex immobilized on the ISNPS silica surface was found to be $\sim 2.0 \times 10^{-4}$ mol g^{-1} and is in agreement with that reported for a similar system [53].

From the results of surface area for ISNPS (Table 1), assuming that the immobilized ruthenium moieties, $[Ru(NH_3)_4(SO_2)]$, are uniformly distributed on the matrix surface, we can estimate an average surface density of 0.45 atom nm^{-2} and a mean intermolecular distance between those moieties of 15 Å. This distance is around 2.5 times the H–N–Ru–N–H mean distances normally observed in similar ruthenium ammine complexes [86,87].

An interesting synthetic approach to preparing the nitrosyl-immobilized ruthenium ammine complex is the use of nitrite aqueous solution at pH 7.4 (*method 2*). It is known that the ruthenium complex with EDTA and iron water-soluble porphyrins [88,89] is able to coordinate to NO_2^-, with subsequent conversion of coordinated nitrite to NO^+. Recently, we found that the aqua complex *trans*-$[Ru(4\text{-acpy})(NH_3)_4(OH_2)]^{2+}$ (4-acpy = 4-acetylpyridine) was able to react with nitrite to generate the corresponding nitrosyl complex in solution, as verified by techniques such as UV–vis, IR spectroscopy, and cyclic voltammetry [90]. As we will discuss later, the immobilized nitrosyl ruthenium complex prepared via reaction with nitrite at pH 7.4 shows behavior very similar to that of the one prepared using NO_2^- in acidic medium (*method 1*).

All the immobilized ruthenium complexes were characterized by UV–vis (Table 3) and infrared spectroscopic techniques.

The UV–vis absorption bands of the immobilized complexes were compared to those of the analogous compounds in solution (Table 3). Two absorption bands are observed for the immobilized complexes. One is in the region 268–274 nm and is probably due to isonicotinamide internal ligand (IL) transitions, since the free ligand and $[\equiv Si(CH_2)_3\text{-isn}]$ show a band in this region. For the ruthenium(II)-immobilized complexes with SO_3^{2-}, SO_2, and H_2O ligands, a second absorption band is seen in the range from 380 to 492 nm. These bands have been attributed [68,94–96] to metal-to-ligand charge transfer (MLCT) Ru(II) → L (L = nitrogen heterocyclic bases) transitions. The immobilized nitrosyl ruthenium ammine showed one absorption band at ∼330 nm, which is similar to the complex in solution. The other absorption bands were not seen in the UV–vis spectra. This is probably due to the matrix absorption that masks the band at 230 nm and the very low molar absorption at 486 nm ($\varepsilon = 4.4 \times 10^1$ L mol^{-1} cm^{-1}) observed for the complex in solution [73]. For the Ru(III) species, $[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(SO_4)]^{3+}$, one absorption was identified at 340 nm, which has been attributed to a ligand–metal charge transfer (LMCT) [92]. Although some shifts were observed for the immobilized complexes, the maximum absorption band values are in agreement with what was observed for the complexes in solution. The shifts observed are possibly due to the slight modification of the isonicotinamide group with the propyl arm since the wavelength and intensity of these bands depend on the ligands located in the *trans* position [68] to SO_3^{2-}, SO_2, and H_2O ligands.

All the immobilized complexes were investigated by IR spectroscopy. Except for the nitrosyl complex, the presence of bands in the IR spectra other than those described for the presence of isonicotinamide groups (Section 3.1) was not observed. This is due to strong bands of the silica matrix, which dominates large ranges of the IR spectra [52,97]. For $[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(NO)]^{3+}$ prepared by *methods 1* and *2*, a very distinct band for both was observed at 1930 cm^{-1} (Fig. 2). This band is very close to the one observed for the solid *trans*-$[Ru(NO)(NH_3)_4(isn)](BF_4)_3$ (1923 cm^{-1}) [73] and by analogy was assigned to $\nu_{(NO^+)}$. As depicted in Fig. 2, both observed stretchings have essentially the same wavenumber, which strongly suggests that the immobilized ruthenium nitrosyl ammine is being formed from reaction with nitrite at pH 7.4. This assumption is supported by cyclic voltammetry data.

Table 4 shows redox potentials for some immobilized ruthenium ammine complexes. In general, the metal-centered redox processes $Ru^{II/III}$ are in agreement with those observed for the analogous complex in solution under similar experimental conditions. For $[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(SO_4)]^+$ at a scan rate of 30 mV s^{-1}, no reverse peak was observed. It is known that the electrochemical processes involving *trans*-$[Ru^{III}(SO_4)(NH_3)_4(L)]^{+/0}$ (L = nitrogen heterocyclic bases) in solution show irreversible behavior due to the fast aquation of sulfate after reduction of the Ru(III) center [98]. This seems also to be the case for the immobilized complex.

Table 3
UV–vis absorptions for the immobilized ruthenium complexes[a] and some related complexes in solution

Complex	λ (nm)[b]
$[\equiv Si(CH_2)_3\text{-isn}]$ isonicotinamide	270, 268[c]
$[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(SO_3)]$	274, 424
trans-$[Ru(isn)(NH_3)_4(SO_3)]$	417[d]
trans-$[Ru(isn)(NH_3)_5]^{2+}$	260, 479[e]
$[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(SO_2)]^{2+}$	270, 380
trans-$[Ru(isn)(NH_3)_4(SO_2)]^{2+}$	268, 356[f]
$[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(SO_4)]^{+}$	264, 340
trans-$[Ru(isn)(NH_3)_4(SO_4)]^{+}$	268, 334[g]
$[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(OH_2)]^{2+}$	274, 492
trans-$[Ru(isn)(NH_3)_4(OH_2)]^{2+}$	266, 486[f]
trans-$[Ru(isn)(NH_3)_4(OH_2)]^{2+}$	485[h]
$[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(NO)]^{3+}$	270, 334[i]
$[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(NO)]^{3+}$	268, 330[j]
trans-$[Ru(NO^+)(NH_3)_4(isn)]^{3+}$	230, 268, 323, 486[k]

[a] CCl_4 suspension.
[b] Uncertainty of ±5 nm.
[c] Ref. [73].
[d] Ref. [85]. Obtained in an excess of ligand, [isn] > 0.1 mol L^{-1}. The UV part of the spectrum is not seen due to free ligand absorption.
[e] Ref. [91].
[f] In this work, obtained with freshly prepared complexes, *trans*-$[Ru(isn)(NH_3)_4(SO_2)]^{2+}$ in 1 mol L^{-1} HCl degassed aqueous solution and *trans*-$[Ru(isn)(NH_3)_4(H_2O)]^{2+}$ in 0.1 mol L^{-1} degassed HCl aqueous solution, respectively.
[g] Ref. [92].
[h] Ref. [93]. Obtained in an excess of ligand, [isn] > 0.1 mol L^{-1}. The UV part of the spectrum is not seen due to free ligand absorption.
[i] Prepared with nitrite at pH 1.
[j] Ref. [73].
[k] Prepared with nitrite at pH 7.4.

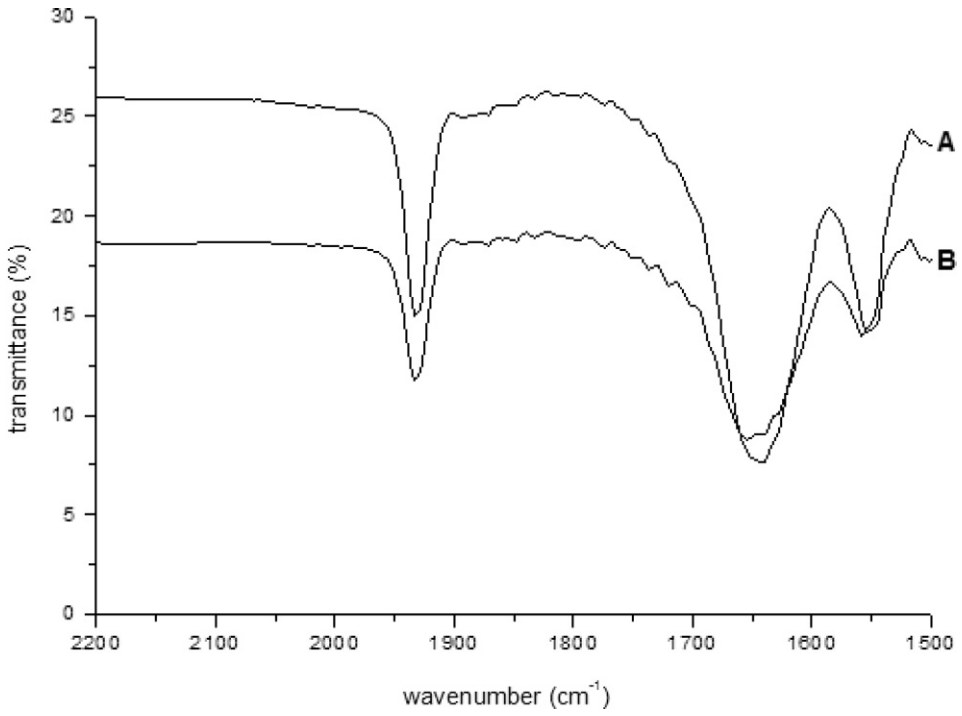


Fig. 2. Infrared spectra of $[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(NO)]^{3+}$ prepared by *method 1* (A) and *method 2* (B) in KBr pellets.

Table 4
Redox potentials for some supported ruthenium complexes and analogous complexes in solution

Ruthenium complex	Potential[a] (V vs Ag/AgCl)
$[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(SO_2)]^{2+}$	0.40[b]
trans-$[Ru(isn)(NH_3)_4(SO_2)]^{2+}$	0.42[b]
$[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(SO_4)]^{+}$	0.025[c]
trans-$[Ru(isn)(NH_3)_4(SO_4)]^{+}$	0.057[c]
trans-$[Ru(isn)(NH_3)_4(SO_4)]^{+}$	0.067[c,d]
$[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(NO)]^{3+}$	0.29[e]
$[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(NO)]^{3+}$	0.27[f]
trans-$[Ru(NO^+)(NH_3)_4(isn)]^{3+}$	0.22[g]
trans-$[Ru(NO^+)(NH_3)_4(isn)]^{3+}$	0.21[h]

[a] In 1.0 mol L^{-1} KCl solution pH ∼ 1, $v = 30$ mV s^{-1}, 25 °C.
[b] $E_{1/2}$ for the $Ru^{II/III}$ couple.
[c] E_{cp} for the reaction $[Ru(SO_4)(NH_3)_4(isn)]^{+} + H_2O \rightarrow [Ru(NH_3)_4(isn)(OH_2)]^{2+} + SO_4^{2-}$.
[d] From Ref. [98]. Value was converted from SCE to Ag/AgCl.
[e] Prepared by *method 1*. E_{cp} for the $NO^{+/0}$ couple.
[f] Prepared by *method 2*.
[g] This work.
[h] $E_{1/2}$ from Ref. [73]. Value was converted from SCE to Ag/AgCl, for comparison purposes.

Nitrosyl ruthenium ammine *trans*-$[Ru(NO)(NH_3)_4(L)]^{n+}$ (L = nitrogen heterocyclic bases) [8,73,86] complexes and others, including those synthesized with chelate ligands such as EDTA [99] and cyclam [100], in solution show a reduction peak that has been assumed to be mainly centered on the nitrosyl group (NO^+/NO^0 process). The immobilized nitrosyl ruthenium ammine prepared from *methods 1* and *2* shows one irreversible cathodic peak ($E_{cp} = -0.29$ V and -0.27 V, respectively) with close potentials as seen from data of Table 4 and as can be seen in Fig. 3. The irreversibility of the process is possibly related to the experimental conditions (scan rate and temperature), since at $v < 0.05$ V s^{-1} in solution for the isonicotinamide, nicotinamide, and pyridine related complexes similar behavior is observed in solution [73,86]. Reversibility in observed only at higher scan rates and/or low temperatures [73,86]. The cathodic peak observed for $[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(NO)]^{3+}$ is close to the observed for the analogous *trans*-$[Ru(NO)(NH_3)_4(isn)]^{3+}$ complex in solution and thus it can be attributed to the NO^+/NO^0 process.

As observed from the results described above, ruthenium ammine complexes immobilized on an ISNPS surface present similar behavior compared to the complexes in solution as observed in xerogel-entrapped *trans*-$[Ru(NO)Cl(cyclam)](PF_6)_2$ complex [39] and in $[Ru(edta)NO]^-$ chemically bound to modified silica gel surface [61].

3.3. Release of NO from the immobilized complex and regeneration of the material

Nitric oxide release from the immobilized complex was investigated by means of light irradiation and chemical reduction.

3.3.1. Photochemical release of NO

Irradiation of ruthenium ammine nitrosyl complexes in aqueous solution with light of energy corresponding to the bands in the range of 300–350 nm results in nitric oxide aquation and formation of the corresponding aqua ruthenium(III) complex [63],

$$\textit{trans}\text{-}\left[Ru^{II}(NO)(NH_3)_4(L)\right]^{3+} \xrightarrow[H_2O]{h\nu} \textit{trans}\text{-}\left[Ru^{III}(OH_2)(NH_3)_4(L)\right]^{3+} + NO. \quad (3)$$

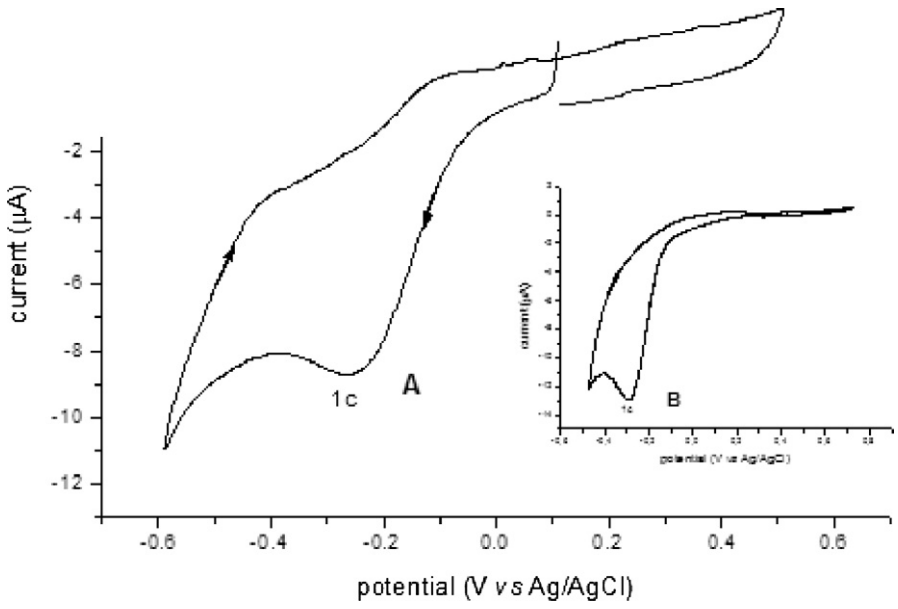


Fig. 3. Cyclic voltammogram of $[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(NO)]^{3+}$ in carbon paste modified electrode in 1.0 mol L^{-1} KCl solution, pH ∼ 1, $v = 30$ mV s^{-1}. (A) Prepared by *method 2*. (B) Prepared by *method 1*.

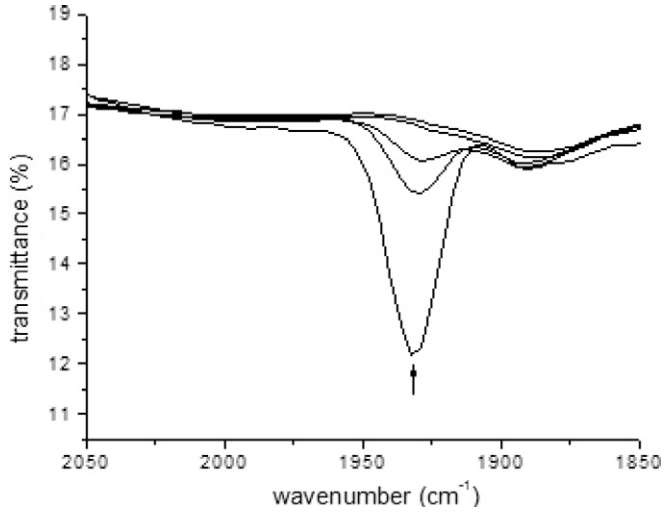


Fig. 4. IR spectral changes during white light irradiation of $[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(NO)]^{3+}$ prepared by *method 2* in a KBr pellet.

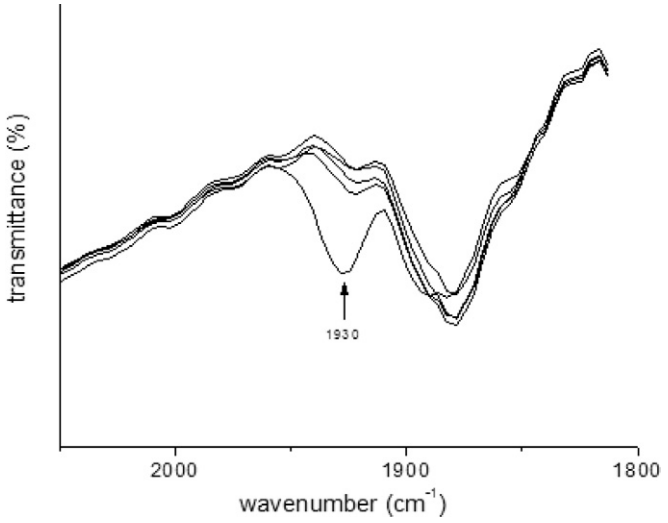


Fig. 5. IR spectral changes during white light irradiation of the immobilized $[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(NO)]^{3+}$ complex after photolysis and regeneration by treatment with aqueous nitrite solution, in KBr pellet.

To investigate if this property is kept after immobilization, ∼1 mg of the $[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(NO)]^{3+}$ was dispersed in a KBr pellet and photolyzed at 334 nm. A very slow decrease of $\nu_{(NO^+)}$ was observed during 24 h photolysis. However, when irradiation was performed with white light, $\nu_{(NO^+)}$ vanishes after around 1 h (Fig. 4).

Photolysis of a KBr pellet containing the immobilized nitrosyl ruthenium ammine complex prepared by *method 1* or the solid *trans*-$[Ru(NO)(NH_3)_4(isn)](BF_4)_3$ under similar conditions displayed the same spectral changes as those observed in Fig. 4. These results suggest that after immobilization NO photolabilization is achieved. Nitric oxide release upon light irradiation was also observed for other systems containing other immobilized ruthenium nitrosyl complexes [37,39,40]. The ruthenium(III) moieties formed after NO photolabilization in silica gel material are possibly associated with water molecules from the silica surface, since the matrix contains typically 1–2% of physically adsorbed water [52,101].

3.3.2. Regeneration of the photolyzed material

In order to explore the possibility of the regeneration of the immobilized complex, the spent material was treated with nitrite aqueous solution. The IR spectra of the resulting solid showed a band at 1930 cm^{-1}, which was observed prior to light irradiation for $[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(NO)]^{3+}$ prepared by *method 2*. This result suggests the presence of nitrosyl coordinated to the ruthenium core. Similar results were found for the $[Ru(salen)(OH_2)(NO)]^+$ complex entrapped in a silica sol–

gel material [40]. The entrapped ruthenium salen complex took 1 week to be regenerated after photolysis, possibly, at least in part, due to slow diffusion of nitrite species into the constrained environment of silicate net. In the case of the ruthenium ammine the surface anchoring makes it prone to be regenerated as fast as the mixing time with nitrite solution, since ruthenium moieties are readily available, thus not requiring nitrite ions to diffuse into the matrix. Fig. 5 shows the IR spectra during the photolysis of the regenerated material. As observed previously, the band at 1930 cm^{-1} vanishes, indicating photolabilization of the coordinated nitrosyl.

3.3.3. Release of NO by chemical reduction

NO aquation upon chemical reduction in the immobilized nitrosyl complex was investigated by IR and UV–vis spectroscopy as well as by using an NO selective electrode. It is known that in solution the complex *trans*-$[Ru(NO)(NH_3)_4(L)]^{3+}$ is quantitatively reduced by europium(II) ions [73] exhibiting a nitric oxide dissociation with formation of the respective aqua species. NO dissociation after reduction in the nitrosyl-immobilized complex was similarly investigated using Eu(II) ions,

$$\textit{trans}\text{-}\left[Ru^{II}(NO)(NH_3)_4(L)\right]^{3+} + Eu^{2+} \xrightarrow{H_2O} \textit{trans}\text{-}\left[Ru^{II}(OH_2)(NH_3)_4(L)\right]^{2+} + NO + Eu^{3+}. \quad (4)$$

Infrared spectra of the solid obtained after reaction of $[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(NO)]^{3+}$ and Eu(II) ions showed complete vanishing of $\nu_{(NO^+)}$. In addition to IR results, the UV–vis spectrum (Fig. 6) showed a band at 486 nm, which is in agreement with that observed for the complex *trans*-$[Ru(isn)(NH_3)_4(OH_2)]^{2+}$ and $[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(OH_2)]^{2+}$ (see Table 3). These results suggest that after reduction with Eu(II) ions, NO is aquated in the immobilized complex, as observed in solution.

Nitric oxide release from the nitrosyl-immobilized complex after reduction with Eu(II) ions was measured with an NO selective electrode. It should be pointed out that addition of a Eu(II) solution gave no significant changes in the current signal measured with the electrode. Fig. 7 shows the chronoamperograms for chemical reduction of $[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(NO)]^{3+}$ (Fig. 7A) and *trans*-$[Ru(NO)(NH_3)_4(isn)]^{3+}$ (Fig. 7B) with Eu(II) ions.

The concentration of NO was calculated as 58 ± 3 μmol L^{-1} and 46 ± 2 μmol L^{-1} for $[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(NO)]^{3+}$ and *trans*-$[Ru(NO)(NH_3)_4(isn)]^{3+}$, respectively, which is in good agreement with the expected calculated values of 60 μmol L^{-1} for $[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(NO)]^{3+}$ and 48 μmol L^{-1} for *trans*-$[Ru(NO)(NH_3)_4(isn)]^{3+}$. These results suggest that NO release after immobilization is essentially quantitative after reduction with Eu(II) ions, as presumed by IR and UV–vis spectroscopy. The results also reflect the possible advantage of immobilizing the complex on the surface of a matrix, which makes it prone to be reduced, since no diffusion of the reducing agent into the polymeric backbone is necessary. The similarity of NO release properties between the immobilized and solution nitrosyl complexes also suggests that nitric oxide release is not significantly affected upon anchoring of the isonicotinamide ruthenium nitrosyl complex.

3.3.4. Regeneration of the chemically reduced material

An interesting property that immobilization could offer is the potential regeneration of the immobilized nitrosyl complexes. Since aqua ruthenium(II) species are formed after reaction of $[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(NO)]^{3+}$ with Eu(II) ions based on the UV–vis spectrum (Fig. 6) and results reported in solu-

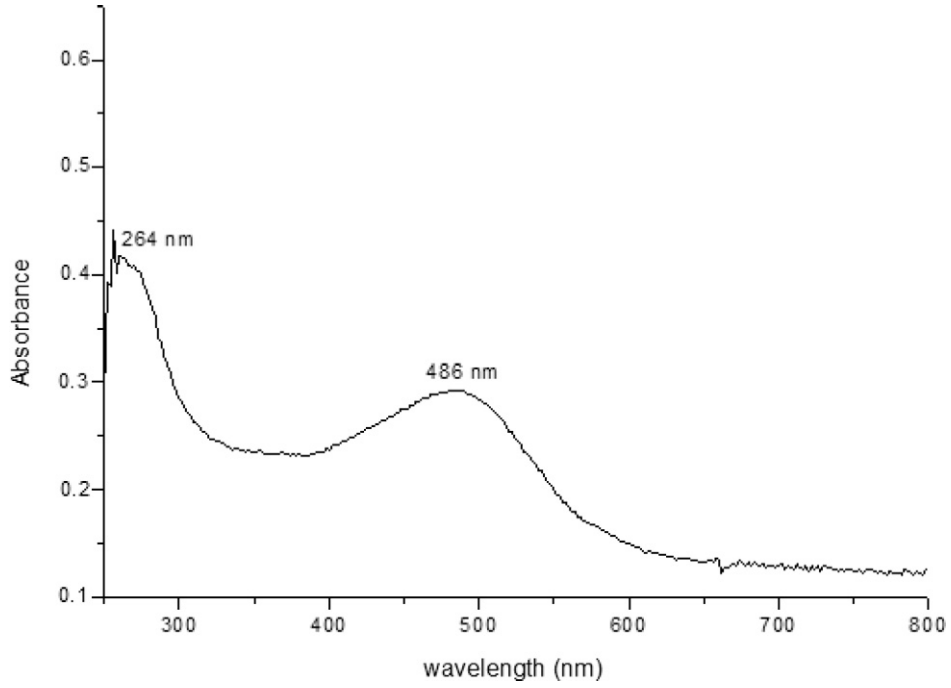


Fig. 6. UV–vis spectrum obtained after reaction of $[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(NO)]^{3+}$ with Eu(II), in CCl_4 suspension.

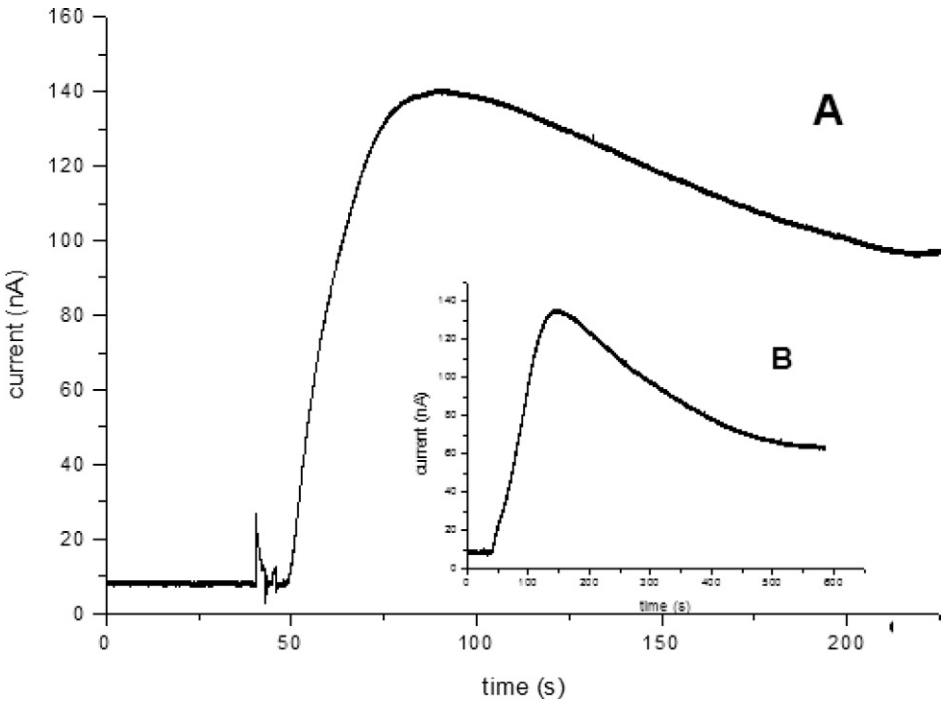


Fig. 7. (A) Chronoamperograms for reaction of 3.0 mg $[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(NO)]^{3+}$ (2.0×10^{-4} mol g^{-1}) suspension with 30 times Eu(II) excess in 10 ml phosphate buffer. (B) 48 μmol L^{-1} solution of *trans*-$[Ru(NO)(NH_3)_4(isn)]^{3+}$ in 10 ml phosphate buffer.

Table 5
Loading of *trans*-$[(isn)Ru(NH_3)_4(NO)]^{3+}$ in the ISNPS support and NO release for the starting and recycled materials

Cycle	"Loading" of complex[a]	NO released ($\times 10^{-4}$ mol) per g of material[b]
0	1.3	1.13
1	1.2	1.01
2	1.0	0.90
3	1.1	1.02

[a] Based on ruthenium elemental analysis according to Ref. [78]. Uncertainty $\pm 0.2 \times 10^{-4}$ mol g^{-1}.
[b] Uncertainty $\pm 0.02 \times 10^{-4}$ mol g^{-1}.

tion [73], we expected that the nitrosyl-immobilized complex could be regenerated by reaction with an aqueous solution of nitrite without significant leaching of the immobilized complex. Three recycling cycles were carried out by reducing the immobilized nitrosyl complex following its regeneration by reaction with NO_2^-. After each cycle ruthenium elemental analysis was performed to evaluate if any leaching had occurred and the solids were analyzed by IR spectroscopy. The IR spectrum of each recycled material as well as the starting material showed the characteristic peak at 1930 cm^{-1} attributed to $\nu_{(NO^+)}$. The release of NO after recycling was evaluated using the NO sensor and the data are collected in Table 5.

Considering the results shown in Table 5, some leaching of the complex (about 15% based on ruthenium elemental analyses of the starting material) seems to occur in the three recycling experiments. This could be due to some leaching of the organosilane groups attached to the silica surface since APS, which is the starting material for ISNPS, is more stable in acidic aqueous solution but loses aminosilane groups in aqueous solution at neutral pH [102]. However, the ratio of NO released/Ru per gram of solid remains essentially constant. This indicates that the immobilized complex can be regenerated without losing its ability as a potential NO-releasing material. From the results presented above, the following reactions for the catalytic cycle with the immobilized isonicotinamide ruthenium ammine complex can be proposed:

$$S\text{–}[Ru\text{–}NO]^{3+} + Eu^{2+} \xrightarrow[H_2O]{} S\text{–}[Ru\text{–}OH_2]^{2+} + NO + Eu^{3+}, \quad (5)$$

$$S\text{–}[Ru\text{–}OH_2]^{2+} + NO_2^- \rightleftharpoons S\text{–}[Ru\text{–}NO_2]^{+} + H_2O, \quad (6)$$

$$S\text{–}[Ru\text{–}NO_2]^{+} + H_2O \rightleftharpoons S\text{–}[Ru\text{–}NO]^{3+} + 2OH^-, \quad (7)$$

$$S\text{–}[Ru\text{–}NO]^{3+} + Eu^{2+} \xrightarrow[H_2O]{} S\text{–}[Ru\text{–}OH_2]^{2+} + NO + Eu^{3+}. \quad (8)$$

4. Summary

The results show that ruthenium tetraammine complexes and particularly *trans*-$[(isn)Ru(NH_3)_4(NO)]^{3+}$ can be immobilized on a modified silica gel surface without loss of their properties, with the last one forming $[\equiv Si(CH_2)_3(isn)Ru(NH_3)_4(NO)]^{3+}$. This material is stable and is able to release NO by reduction with Eu(II) or photochemically, resulting in the respective immobilized Ru(II) and Ru(III) aqua species, respectively. The NO-depleted material can be regenerated to its original nitrosyl form by reaction with nitrite at physiological pH. There is no significant leaching from the material up to the three regeneration cycles studied. The immobilization of these complexes shows an interesting strategy for preparing new NO-releasing

materials. This approach is quite useful since immobilized nitrosyl ruthenium ammines with different ligands could be prepared, resulting in different NO-releasing properties, since the properties of ruthenium amines that lead to NO release can be tuned by an adequate choice of ligands. The NO release can then be triggered by an adequate choice of reducing agents or irradiation wavelength, with rates dependent on the ligands or the medium. The conversion of nitrite into coordinated nitrosyl with good chemical stability presented by the supported complex could also open a new possibility for the preparation of Ru-based materials that are able to generate a constant NO concentration. The possibility of regenerating the NO-depleted material, obtained either by photolysis or chemical reaction, by reaction with nitrite shows an interesting approach since NO_2^- is the largest pool of NO_x species present in the blood stream and may serve as a potential source of NO. Thus, immobilization of nitrosyl ruthenium complexes may allow the use of nanoparticulated silica gel as a drug carrier or as part of a cover layer for implant devices with the aim of exploring NO releasing properties. The results obtained in this work can contribute to the design of NO releasing materials with the desired properties.

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