Cyclodextrins in Nitrosation Chemistry: New Insights of the NO-Transfer Processes

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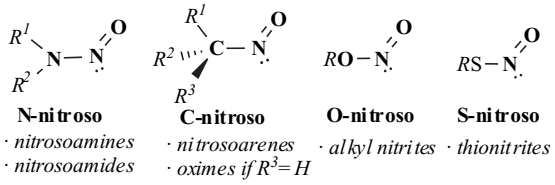
Abstract: The chemistry of nitroso compounds has received considerable attention from several directions in recent years. This review will focus on describing the practical aspects, and current and potential applications of cyclodextrins in mechanistic studies of NO-transfer processes. The first section describes some physico-chemical aspects of aqueous cyclodextrin solutions that are of crucial importance in relation to mechanistic studies. The next section analyses all types of information that can be obtained from different nitrosation reaction patterns either in acid or basic media, by paying special attention to the nitrosatable substrates and nitrosating agents. Relevant aspects concerning the biological activity of nitroso compounds is also included. The last section covers a detailed analysis of the effects of cyclodextrins on reactions undergone by nitroso compounds, such as hydrolysis or NO-transfer, as well as on reactions that produce nitroso compounds.

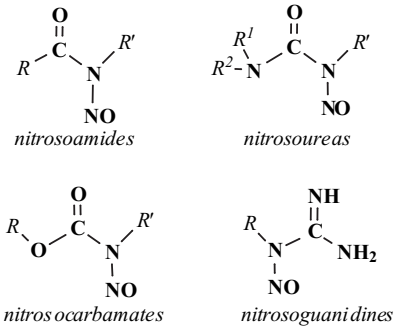
Keywords: Nitrosation, NO-transfer, cyclodextrins, reaction mechanism, catalysis.

1. INTRODUCTION

Nitroso compounds occupy a prominent position in organic chemistry as both useful synthetic intermediates and molecules of biological interest [1]. The chemistry of organic nitroso compounds is in many ways linked to the chemistry of nitric oxide (NO) [2,3]. Diverse organic substrates including amines, amides, amino-acids, enols, phenols, thiols, …, may be converted in nitroso compounds of general structure Y-N=O (Y= N, C, S, O donors, Scheme **1**) [4], that exhibit different chemical and biological reactivity.



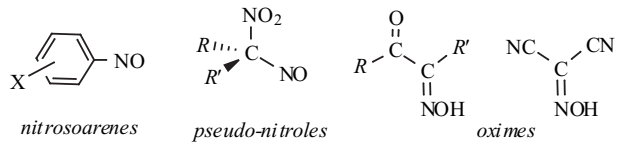
Scheme 1. Representative organic nitroso compounds.



Scheme 2. Representative structures of N-nitrosoamides.

N-nitroso compounds can be divided into N-nitrosamines –derived from dialkyl, alkylaryl, diaryl, or cyclic secondary amines–, and N-nitrosoamides –generated from N-alkylureas, N-alkylcarbamates, and simple N-alkylamides; other N-nitroso compounds can also be made from cyanamides, guanidines, hydrazines, amidines (Scheme **2**). Monoalkylnitrosamines formed from primary aliphatic amines are unstable and decompose to the corresponding alcohol and olefins.

C-Nitroso compounds can be divided into nitrosoarenes or aromatic C-nitroso compounds (*e.g.* nitrosophenol, 1-nitroso-2-naphthol) and aliphatic C-nitroso compounds that are mainly derived from aliphatic nitro compounds, and ketones or nitriles, to give pseudo-nitroles or oximes, respectively; that is, C-nitroso aliphatic compounds are obtained when strong electron-withdrawing groups are attached to the C-atom, Scheme **3**. The nitrosation of 3-substituted indoles yield also C-nitroso compounds.



Scheme 3. Representative C-nitroso compounds.

Alkyl nitrites (or O-nitroso compounds) and thionitrites (or S-nitroso compounds) are obtained in the nitrosation of aliphatic alcohols or thiols, respectively.

Nitrosation reactions have been much used synthetically and many aspects of nitroso compounds have been investigated from several viewpoints.: 1) Some of these substances –in particular, N-nitrosamines– have proved to be powerful carcinogens in all animal species which have been tested with [5]. The toxic, carcinogenic, mutagenic, and teratogenic effects of some nitroso compounds are now well documented. 2) Since 1987, when it has been demonstrated the enzymatic formation of NO from L-arginine and the necessary role of NO (nitric oxide) in the vascular muscle relaxation [6], an explosion of activity on the chemistry of NO including all NO-precursors (alkyl nitrites, alkyl

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nitrates, nitroso compounds, …) has been developed. 3) To a complete knowledge of the factors that affect the NO-transfer processes, the kinetic study of nitrosation reactions has proved to be a fruitful one from the mechanistic point of view. The NO-transfer can occur at carbon, nitrogen, oxygen, sulphur, and halogen atoms, as well as with transition-metal-complexes. C-Nitroso compounds –or oximes, when possible–, N-nitrosamines, alkylnitrites, thioalkylnitrites, nitrosyl halides and nitrosyl-metal complexes such as sodium pentacyanonitrosylferrate (II), have been devoted to many studies that consider different aspects of these NO-precursors. The nitrosation reactions have been largely studied in water, organic solvents, mixtures of water and organic solvents, and, in the last few years, also in microheterogeneous media, particularly in aqueous solutions of surfactant forming micelles and of cyclodextrins.

The aim of this review is to describe the practical aspects, and current and potential applications of cyclodextrins in mechanistic studies that involve nitrosation reactions particularly. The first section describes physico-chemical aspects of aqueous cyclodextrin solutions relative to mechanistic studies. The next section characterizes the types of information in different nitrosation reaction patterns that are usually obtain in acid and basic media, by paying special attention to the nitrosatable substrate and nitrosating agent. The last section focuses on the effect of cyclodextrins on reactions undergone by some nitroso compounds and on some nitrosation reactions that produce nitroso compounds.

2. CYCLODEXTRINS

The discovery of cyclodextrins (CDs) by Villiers in the late 19th century [7] was soon followed by their preparation and isolation by Schardinger in the early 1900s [8]. Since that time, continued scientific interest has led to the exploration and development of a variety of novel chemical applications for these seductive and promising molecules [9].

2.1. Properties of CDs

Cyclodextrins [10-12] comprise a family of cyclic oligosaccharides built up by glucopyranose units joined by $\alpha(1\rightarrow4)$ glycosidic linkages involving axial C_1-O and equatorial C_4-O bonds. The best characterized structures are α-, β-, and γ-cyclodextrin formed with six, seven and eight-D-glucopyranose units, respectively. The common molecular structure of these three forms is truncated cone-shape with a hallow tapered cavity of 7.8 Å depth equal for the three major forms, Fig. **1**.

The number of D-glucopyranose units defines the width of the central cavity and the flexibility of the compound. As a consequence of the $^4C_1$ conformation of the glucopyranose units, all secondary hydroxy groups are situated on one of the two edges of the ring, whereas all the primary ones are placed on the other edge. The cavity is lined by the H-atoms and the glycosidic oxygens bridges (-O-). The nonbonding electron pairs of the glycosidic oxygens are directed towards the inside of the cavity, producing there a high electron density and lending to it some Lewis base character. The C-2 OH-groups of one glucopyranose unit can form a H-bond with the C-3 OH-group of the adjacent glucopyranose unit. In the β-CD molecule a complete secondary belt is formed by these H-bonds, so that β-CD is a rather rigid structure. This probably explains the observation that β-CD has the lowest solubility of all CDs (Table **1**) since in the α-CD molecule the H-bond belt is uncompleted and the γ-CD molecule has, a non-coplanar, more flexible structure.

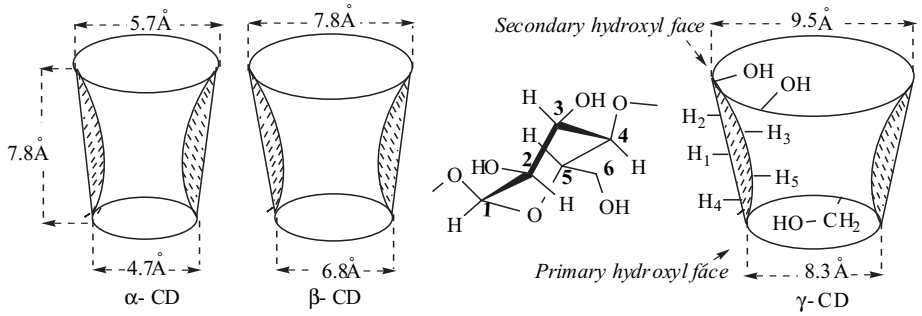


Fig. (1). Molecular shape and dimensions of the three major CDs forms.

Table 1. Some Physicochemical Properties of α-, β-, and γ-Cyclodextrin

Parameter	α-CD	β-CD	γ-CD
Nº of glucose units	6	7	8
M_W (molecular weight)	972	1135	1297
Solubility in water (g/L at ~20 ºC)	145	18.5	232
Approximate cavity volume ($Å^3$)	174	262	427
Relative permittivity (ϵ)[(a)]	47.5	52.0	70.0
pK_a (by potentiometry) at 25 ºC	12.3	12.2	12.1

[(a)] Relative permittivity or dielectric constant of the cavity on incorporating the toluidinyl group of 6-*p*-toluidinylnaphthalene-2-sulfonate at pH 5.3 and 25 ºC.

These three major CDs are crystalline, homogeneous and non-hygroscopic substances naturally obtained during the degradation of the linear amylose component of starch in the presence of enzymes. They have no defined melting points, but from ~200 ºC they begin to decompose. The viscosity of an aqueous CD solution does not differ significantly from that of water. Aqueous CD solutions are transparent to UV and vis light, even though they scatter light at high CD concentration due to the size of these macromolecules. In alkaline media, a secondary OH group is ionized. The pK_a values measured by potentiometry are given in Table **1**.

2.2. Complexation by CDs

The most important feature of cyclodextrins is their hydrophobic cavity, which enables them to form non-covalent inclusion complexes, *i.e.* entities comprising two or more molecules in which one of the molecule –the 'host'– includes, totally or in part, only by physical forces, a 'guest' molecule [13-16]. The most probable mode of binding involves the insertion of the less polar part of the guest molecule into the cavity, while the more polar –or often charged– group of the guest is exposed to the bulk solvent near the secondary hydroxyl rim, *i.e.* outside the wider opening of the cavity. Geometrical factors, such as size and shape, rather than chemical factors are decisive in determining the kind of guest molecules which can penetrate into the CD-cavity. Stabilization of the complex is achieved by van der Waals forces, hydrogen bonding, decrease of strain energy and release of high-energy water from the cavity. The rate constant of inclusion complex formation is practically diffusion controlled; the inclusion process seems to be analogous to the establishment of the enzyme-substrate bond. In solution, complex formation is described as an equilibrium between a substrate S and the CD, eq. **1**, with K_c being the stability constant of the complex. The 1:1 and 1:2 guest:host stoichiometry complexes are the most common types of interaction, but complexes with stoichiometries 2:1, 2:2, or 1:3 have also been reported.

$$CD + S \underset{k_d}{\overset{k_i}{\rightleftharpoons}} CD{\cdot}S \quad K_c = k_i/k_d \tag{1}$$

The enthalpy (ΔH) values of inclusion are always negative, that is, the complex dissociates when the temperature is increased, and the entropy (ΔS) values can be positive or negative. If ΔH is small (little heat is liberated during complexation) the ΔS value is large (high degree of disorder after complex formation); conversely, if ΔH is large (strong host-guest interactions) the ΔS is negative, and complex formation will result in a higher order of the system.

The driving forces leading to the inclusion complexation of CDs should include the electrostatic interaction, van der Waals interaction, hydrophobic interaction, hydrogen bonding, and charge-transfer interaction. However, due to enthalpy-entropy compensation, release of conformational strain and exclusion of cavity-bound high-energy water are not energetically contributive to the complex formation, and the enthalpy and entropy changes of the complexation are not good criteria to be used in judging whether a particular driving force is present or important. For example, the ion-dipole interactions should be enhanced when the charge of the ion increases; thus it can be expected the dianions such as SO_4^{-2} and CO_3^{-2} bind more tightly with CDs than single anions such as ClO_4^-, NO_3^-, Br^-. However, though the complexation of CDs with ClO_4^-, NO_3^- or Br^- has been observed experimentally [17,18], no complex formation can be detected for SO_4^{-2} and CO_3^{-2}.

Changes in spectroscopic and physico-chemical properties, as well as in the reactivity, of the guest accompany the inclusion complexation process. These changes are used for a quantitative determination of the stability constant of a complex. In this sense, complex formation by a CD increases the apparent solubility of a slightly soluble substrate (guest) and this phenomenon can be used to estimate the stability constant. The absorption spectrum of a guest may show significant change when it complexes with a CD –the λ_{max} is shifted or the absorption

Table 2. Stability Constants of 1:1 Inclusion Complex of Host:Guest (CD:S) and Thermodynamic Properties Measured in Water at 25 ºC [16]

Guest / Host	K_c/M^{-1}	ΔH [a]	ΔS [b]	method	K_c/M^{-1}	ΔH [a]	ΔS [b]	method
	α-cyclodextrin (α-CD)				*β-cyclodextrin (β-CD)*			
Benzene	31.6	-13.1	-15.1	vap	107	-3.5	-24.2	cal
Phenol	1585	-7.5	54.4	uv	2512	-11	30.2	cal
Benzoate	10.5	-16.3	-35.2	pot	36.2	-4.6	14.1	pot
$CH_3(CH_2)_4CH_2OH$	891	-19.0	-8.05	uv	219	0.4	46.0	uv
$CH_3(CH_2)_4CH_2NH_3^+$	383	-17.6	-9.73	cal	64.6	2.5	43.3	cal
Cyclohexanol	64.6	-14.0	-14.1	uv	501	-10.0	17.1	uv
Cyclohexane	9	---	---	---	156	---	---	---
ClO_4^-	45.7	-26.4	-56.4	pot	12[c]/27	-16.5	-34.6	cal
SCN^-	34.6	-28.5	-66.4	pot	10	---	---	---

[a] in kJ/mol; [b] in $J{\cdot}mol^{-1}K^{-1}$; [c] in 0.2 M NaCl; vap, vapor pressure; uv, UV-spectroscopy; pot, potentiometry; cal, calorimetry.

intensity is increased/decreased–. Fluorescence measurements can also be applied to the determination of stability constant, whose phenomenon is based on the proportionality of fluorescence intensity to fluorophore concentration. NMR-spectroscopy is capable of providing direct evidence of inclusion within the CD cavity; significant shifts in the ^1H NMR signals of the inside protons H_3 and H_5 of CD are observed upon complexation, along with in the specific ^1H signals of the guest, so it has mainly been used to study not only the structure of the complex, but also the stability constant. The chromatographic mobility of the guest is modified, because the hydrophobic guest upon complexation becomes hydrophilic. The reactivity of the included guest is modified; in this sense, the CD behaves as an artificial enzyme by regulating chemical and photochemical reactivity of the guest. Table **2** reports some representative data of stability constants of CD-inclusion complexes.

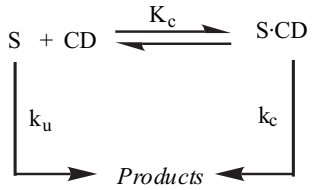
2.3. Effects on Reactivity

The effects of cyclodextrins on organic reactions are divided mainly into two types [19-23]. The first type considers the situation when the substrate (S) forms inclusion complexes with a CD by true covalent bonds. In this case the reaction proceeds according to the Michaelis-Menten type, and the catalytic effect is reported as the '*enzyme model*'. In alkaline medium, a secondary –OH group of the CD is ionized; then, this alkoxide ion acting as a nucleophile, can react with the bound substrate. For example, the esters bound to the cyclodextrin cavity could acylate the alkoxide group, $-O^-$, on the CD wider rim with some geometrical preferences: the hydrolysis of *m*− nitrophenylacetates increases about 100-times in the presence of β-CD [24]. The second effect does not involve a covalent bond. The hydrophobic cavity of the CD gives the reactant access to a new reaction environment, in which the reactivity, such as rate or selectivity, changes. In these cases, it is said that CD *mediates* the reaction. The driving forces leading to the inclusion complexation of CDs are quite variable.

Covalent Catalysis

If one considers that S reacts *via* an *uncatalyzed* (k_u) reaction and a *catalyzed* (k_c) reaction through a *1:1 complex* with a CD, the reaction mechanism of Scheme **4** gives rise to eq. **2**.

$$k_o = \frac{k_u + K_c k_c [CD]}{1 + K_c [CD]} \qquad (2)$$



Scheme 4. Mechanism of CD-catalysis.

The analysis of the experimental data provides the constants k_u, k_c, and K_c, from which it is possible to estimate the most important factor in catalysis, i.e. the stabilization of the transition state (TS) of the reaction by the catalyst. Following the method devised by Kurz [25-27], the quantity K_{TS}, which is the apparent stability constant of transition state formation, define as $K_{TS}=[S\cdot CD]^{\ddagger}/\{[S][CD]\} = (k_c K_c/k_u)$, provides a useful measure of the relative energies of the TS for the catalyzed and the uncatalyzed reactions, under standard conditions, regardless of their actual structures. The overall change observed in the reaction rate is determined by the strength of binding of TS (K_{TS}) relative to that of the substrate (K_c). On the other hand, in covalent catalysis the values of K_{TS} show high sensitivity to structural changes in the substrate due to the strong interaction of the S with the CD and to stringent geometric requirements of the substrate. The other quantity of interest is the second-order rate constant for the reaction between the S and CD, which is determined as $k^2=k_c K_c$. This derived constant measures the substrate selectivity of the cyclodextrin, that is, k^2 measures the ability of CD to select between derivatives of a given reactant, in other words, it analyses the effect of substituents/geometry of substrates in the binding process, Fig. **2**.

Non-Covalent Catalysis

In this case, inclusion of the S into the CD cavity provides a different environment for the reaction. The apparent polarity of the CD cavity seems to depend on the probe used [28]; however, some studies have suggested a similarity to dioxane [24], while others favor ethanol [29]. Catalysis may arise from the less polar nature of the cavity –dielectric effect–, from the conformational restraints imposed on the substrate by the geometry of inclusion, or as a result of differential solvation effect at the interface of the CD-cavity with the bulk, normally aqueous, medium [30]. The non-covalent catalysis is often observed in reactions exhibiting large solvent dependence. Typical characteristics of the non-covalent catalysis are: the effect of CD is comparable to that measured in water-solvent mixtures and significantly less that those measured in wholly organic solvent; values of K_{TS} –the binding of the TS to CD– do not vary greatly with the substituents on the substrate, neither from one CD to another, while, in contrast, K_c –the binding of substrate to CD– changes significantly from one CD to another; modest CD effects (catalysis or inhibition) are observed, and finally, the activation parameters for the CD-mediated reaction are very similar to that determined in a solvent of similar polarity that the CD-cavity interior. Reactions such as intramolecular acyl transfer, decarboxylation of activated carboxylate anions [31], or bromination of phenols [32] are typical examples of non-covalent catalysis.

In general, inclusion catalysis reveals several characteristics of enzyme-catalyzed reactions, such as *saturation limit*, *competitive inhibition* and *unproductive bonding*. Formation of a productive inclusion complex enhances the reactivity of the guest, whereas unproductive complex formation means that the complexed guest is effectively protected against any transformation, except the reaction with hydroxyls of CD.

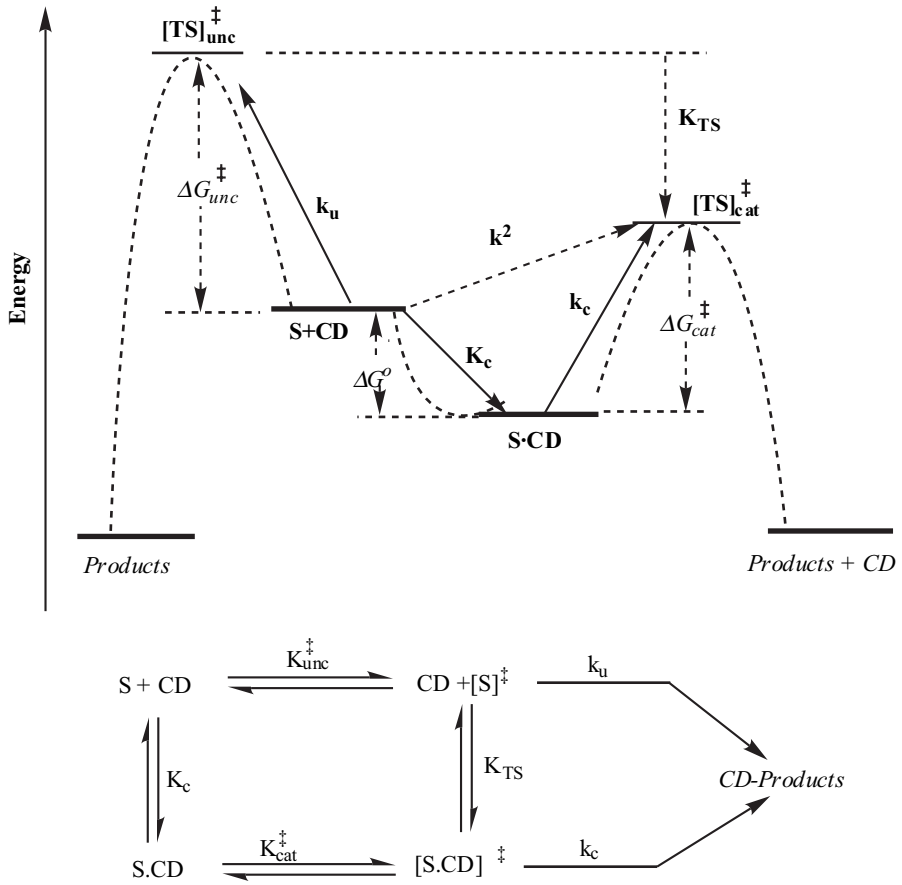


Fig. (2). Energy diagram for both CD-catalyzed and uncatalyzed reactions of a substrate S and the corresponding thermodynamic cycle.

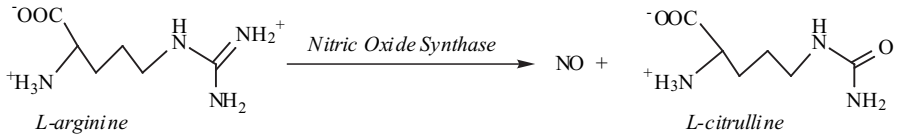
3. NITROSO COMPOUNDS

Reactions that involve the formation and decomposition of nitroso compounds have largely been studied since the early years. Many nitrosation reactions are now standard procedures, both in the laboratory and on the industrial scale, that provide different routes in synthesis. Certain nitroso compounds such as alkyl nitrites, metal nitrosyl complexes or thionitrites are used in medicine as vasodilators or regulators of blood pressure, but others specially nitrosamines are powerful carcinogens. N-Nitrosamines, C-nitroso compounds, alkylnitrites, thionitrites, nitrosyl halides and nitrosyl-metal complexes such as sodium pentacyanonitrosylferrate (II) have been devoted to many studies that consider different aspects of these NO-precursors. Several features such as acid-base catalysis, nucleophilic catalysis, diffusion-controlled processes, isotope effects or intramolecular rearrangements have all been established in nitrosation reactions, which have been largely studied in water, organic solvents, mixtures of water and organic solvents, and, in the last few years, also in microheterogeneous media, in particular, aqueous solutions of surfactant forming micelles and of cyclodextrins. In this section, I shall make a survey of some important aspects of nitrosation chemistry.

3.1. Biological Activity of Nitroso Compounds

In recent years the identification of the physiological activity of nitric oxide (NO) has generated much interest in the chemistry of nitroso compounds or of NO-related bonding. The Nobel Prize in 1998 was earned by Furchcott, Ignarro, and Murad for the discovery of the multiple and exciting roles that NO plays in physiological and pathophysiological functions in humans [33-35]. Nitric oxide is a widespread biological mediator that not only represents the pharmacologically active species of nitrovasodilator drugs such as thionitrites, alkyl nitrites, alkyl nitrates, etc, but is also produced by vascular endothelial cells to regulate blood flow and thrombosis. A variety of cells are capable of biosynthesizing NO *via* the five-electron oxidation of one of the terminal guanidinium nitrogens on the amino acid arginine (Scheme **5**).

The physiology of endogeneous NO generation has been the subject of high interest, due to its role in the vascular system as a vasodilator and inhibitor of platelet function and the central and peripheral nervous systems. NO also plays a major role in host defense mechanisms such as acute inflammation and host response to invasion by bacteria, viruses, and parasites. In these systems, much of the biological activity of NO is due to its direct action on the enzyme guanylate cyclase, *via* its coordination to the enzyme-bond ferrous heme. Therefore, NO has many potent biological activities in organisms, and is characteristically present at extremely low levels. For bioregulatory purposes, NO concentrations less than 1 μM have been reported to be generated in endothelium cells for blood pressure control [36]. Local high concentrations of NO are toxic to living tissues [37, 38].



Scheme 5. Biosyntesis of NO from L-arginine oxidation by the enzyme nitric oxide synthase.

NO is rapidly destroyed by biochemical oxidants such as oxyhemoglobin, oxygen, and superoxide ion. The radical NO· is capable of rapidly reacting with other biologically relevant radicals such as molecular oxygen and superoxide, O_2^-. The oxidation of NO gives N_2O_3, eq. **3**, which is a nitrosating agent [39]. The combination of NO and superoxide gives peroxynitrite, eq. **4**, at rates approaching the diffusion limit ($k \sim 5.5 \pm 1.2 \times 10^9$ $M^{-1}s^{-1}$) [40, 41]. This anion, of very short half life (~1 s), decomposes readily under acid catalysis to either isomerization to nitrate (the major decay route) or by producing a species with reactivity akin to that of ·OH.

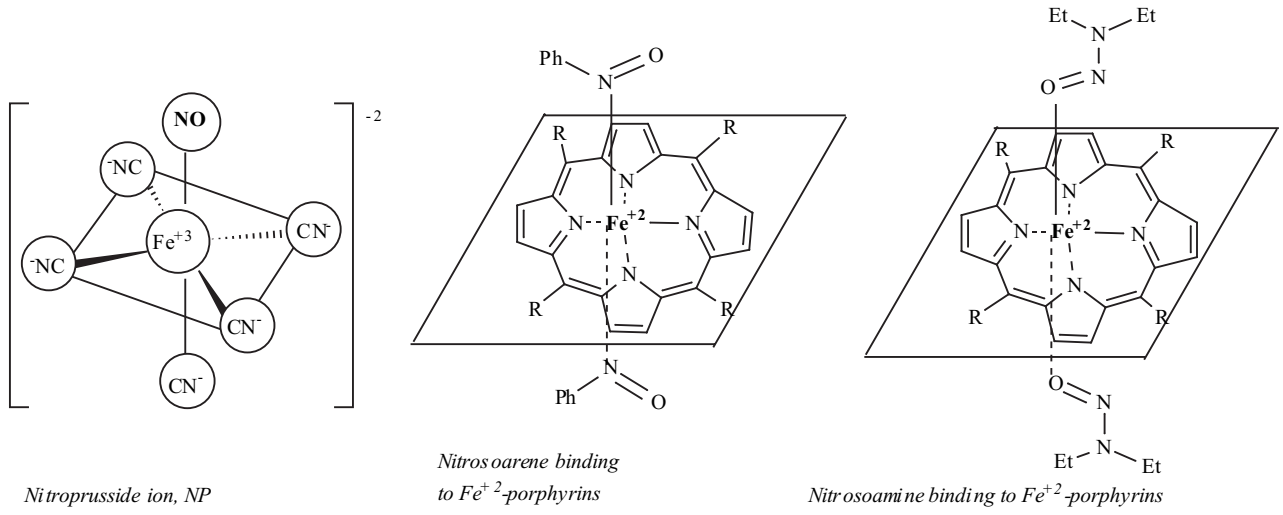
$$NO. + {}^{+1}/_{2}O_2^- \longrightarrow NO_2 \xrightarrow{+NO} N_2O_3 \quad (3)$$

$$NO. + O_2^- \xrightarrow{k} {}^-OONO \longrightarrow NO_3^- \quad (4)$$

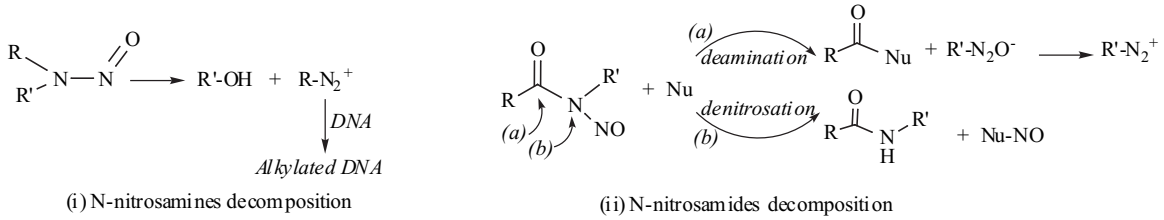
In the same sense, NO and related N_xO_y compounds affect nitrosation of organic substrates: N_2O_3 may damage DNA through either direct nitrosation of primary amines on DNA bases or *via* nitrosation of secondary amines to form N-nitrosamines, some of them can be metabolized to produce strongly alkylating electrophiles that react with DNA; N_2O_3 may also react with essential sulfhydryl groups of various proteins [42, 43]. Nevertheless, the cellular damage caused by high levels of NO is not only due to its action as an oxidant but also NO is a ligand capable of binding vital metal centers [44, 45].

NO is a powerful ligand to metal ions, with binding constants often much higher than those of CO and O_2, and dominates the coordination sphere of the metal [46]. The most widely studied metal nitrosyl complex is sodium nitroprusside ($Na[Fe(CN)_5NO]$, **SNP**); however the coordination complexes of ruthenium and osmium analogs of nitroprusside are also well-known and exhibit behavior similar to that of their iron congener. Metal nitrosyl complexes may be NO donors; in fact SNP has been used clinically to reduce blood pressure, *e.g.*, in hypertensive emergencies. In biological systems, NO release from SNP may occur both enzymically and nonenzymically, requiring the presence of vascular tissue or catalyzed by reducing agents, such as thiols. The latter one-electron reduction process produces significant amounts of NO.

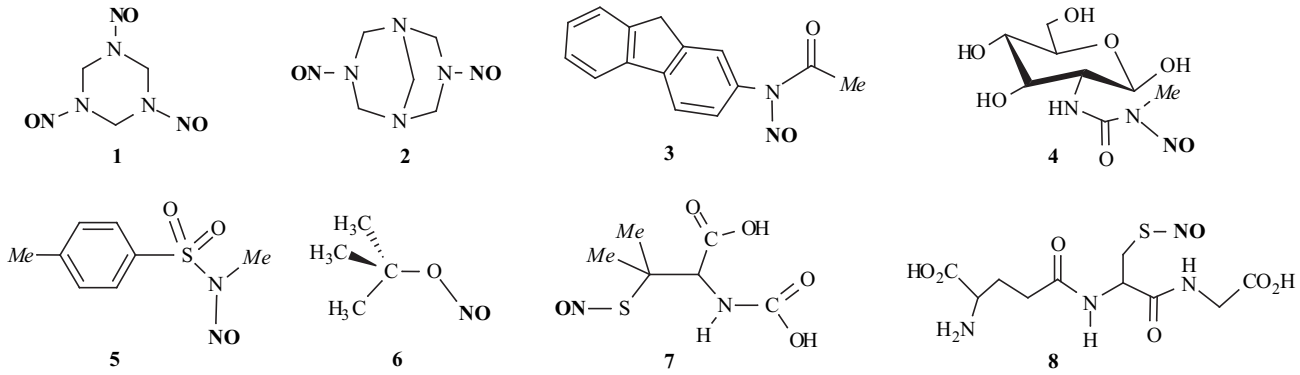
In the same manner, S-nitroso and O-nitroso compounds have proved to interact with the metal centers in metalloporphyrins *via* the non-nitrosyl hetero-atoms, *i.e.* by releasing NO, whereas, N-nitroso and C-nitroso compounds form discrete adducts with metalloporphyrins *via* the interaction of either N- or O-atoms with the metal centres, Scheme **6** [47].



Scheme 6. Modes of binding of NO and nitroso compounds to metal centres.



Scheme 7. Mean reaction pathways of decomposition of both N-nitrosoamines and N-nitrosoamides.



Scheme 8. Molecular structures of representative NO-donors.

Some nitroso compounds decompose or can be metabolized *in vivo* to form strongly alkylating electrophiles that may damage DNA, in which process the cytotoxic and mutagenic effects of carcinogenic compounds are believed to reside. It has been showed that dimethylnitrosamine is converted enzymically into a methylating agent [48] and this N-nitrosamime induces liver cancer when fed to rats [49].

Nitroso derivatives of secondary amines decompose spontaneously *in vivo* leading to the formation of alkylating agents, whereas nitrosamides give either de-amination or de-nitrosation products in the presence of nucleophiles, Scheme **7**.

In other sense, certain nitrosamines (**1, 2** of Scheme **8**) have been proved to inhibit thrombus formation in arterioles and venules of rats, while *e.g.* the nitrosamine **3** exhibits vasodilation and mutagenicity. The nitrosourea **4** (streptozocin) is an antibiotic with diabetogenic, carcinogenic and antitumor activity. The N-methyl-N-nitroso-p-toluenosulfonamide (**5**, MNTS) was found to be a potent antimicrobial agent against fungi as well as Gram-positive and Gram-negative bacterial strains. N-nitrosoguanidines have showed vasodilatation properties *in vitro* and blood pressure lowering effects *in vivo*.

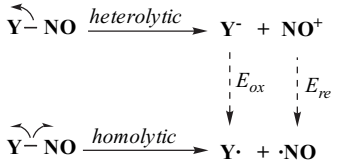
The mechanisms whereby N-nitroso compounds may produce alkylating carbonium ions *via* liberation of nitrogen cannot apply to C-nitroso compounds. However, pseudo-nitroles display good *in vitro* vasodilation, exhibit clear cardiovascular effects or show up to 25% inhibition in the aggregation of blood platelets [50].

Alkyl nitrites used as inhalants cause vasodilation, increase heart rate, and decrease systolic blood pressure [51-53]. Amyl nitrite and *n*-butyl nitrite **6** have been commonly used in relieving angina pectoris. The clinical use of some of these O-nitroso compounds with vasodilation effects are well known.

The biological activity of thio-alkyl nitrites, RSNOs, were known even before that of nitric oxide. In fact, many reports revel the unique relationship between thionitrites and NO. RSNOs themselves can be used as potent antiplatelet agents and vasodilators, whose functions are attributed to NO release. S-Nitroso-N-acetyl-penicillamine, **7** (SNAP), and S-nitroso-glutathione, **8** (GSNO), where also shown to protect the long epithelium from oxidant –induced increases in monolayer permeability- as well as the protection of endothelial cells from the toxic effects of oxidized low density lipoprotein [54]. S-nitroso-hemoglobin represents an oxygen-sensitive modulator of vascular tone [55].

3.2. Transfer of ·NO / NO^+ Groups

For medicinal purposes it is desirable to find NO-donating compounds with well-controlled release. *A priory*, any nitroso compound could be a possible candidate donor of NO. Two possible modes of cleavage of the Y-NO bond of nitroso compounds, that is, *homolytic* and *heterolytic* cleavages are well recognized (Scheme **9**) giving rise to nitric oxide (NO) or nitrosonium ion (NO^+) donors, respectively.

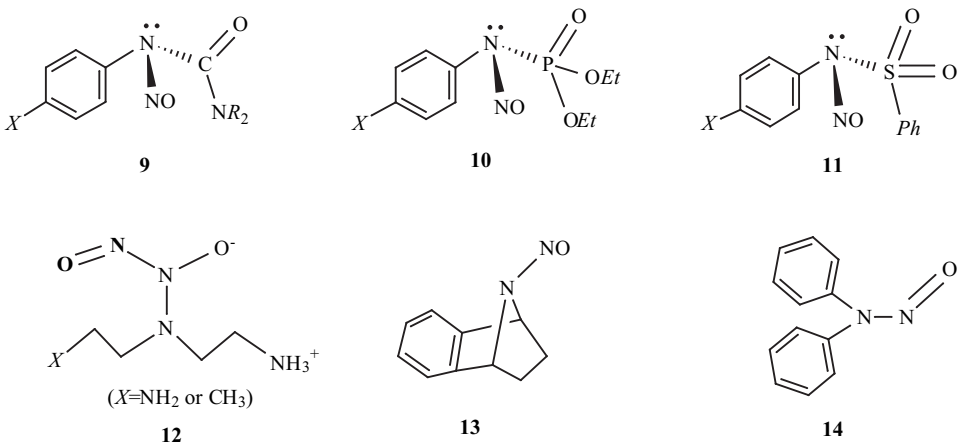


Scheme 9. Two possible modes of Y—NO bond cleavage of nitroso compounds.

The binding force of NO with a particular active site can be estimated by the bond energy of the Y—NO type, where Y is a relatively large organic moiety. Following to Arnett [56] and Bordwell [57,58] the difference between heterolytic bond energy (ΔH_{het}) and homolytic bond energy (ΔH_{hom}) is closely approximated to the enthalpy of electron transfer, that is, ΔH_{het} can be obtained from the heat of combination reaction between Y^- and NO^+ measured in solvents like acetonitrile or dimethylsulfoxide, whilst ΔH_{hom} is obtained from ΔH_{het} according to eq. **5** where E_{re} is the reduction potential of NO^+ and E_{ox} represents the oxidation potential of Y^-.

$$\Delta H_{hom} = \Delta H_{het} - 23.06\ (E_{re} - E_{ox})\ \text{in kcal/mol} \qquad (5)$$

Based on this method analysis, N-nitrosoureas **9** and N-nitrosophosphoramides **10** –given in Scheme **10**– have ΔH_{het} (about 50-62 kcal/mol) almost double the values determined for N-nitrososulfonamides **11** (of 25-35 kcal/mol), and for the three kind of compounds Y—NO homolysis energies are substantially lower than the corresponding heterolysis energies. The consequence is that, whereas N-nitrosoureas react with nucleophiles *via* the attack at the carbon of C=O to generate alkylating species which account for their anti-cancer activity [59], N-nitroso-sulfonamides react with nucleophiles preferentially by



Scheme 10. Molecular structures of typical NO donors.

denitrosation pathway to release NO^+ and result in transnitrosation reactions [60].

The Griess assay is a useful method to evaluate release of ·NO or NO^+ transfer from nitroso compounds in solution [61]. The method is based in the measurement of the visible absorption at 595 nm of the resulting dye formed upon diazo coupling of the Griess reagents after reacting with NO/NO^+. A recent study has used this method to evaluate NO/NO^+ release from several nitroso compounds [62]. The results shown that compounds **7**, **8**, or **12** can be considered as *authentic NO donors*. The substrate **12** gives a rapid and steady formation of the dye and the rate of the process is insensitive to the pH of the medium, whereas S-nitroso-N-acetyl penicillamine **7** shows also rapid formation of the dye but the rate is acidity-dependent and the dye concentration increases with time. By contrast, monocyclic-aliphatic-N-nitrosamines (*cyclo*-$H_{2n}C_nN$-NO, n=3 to 7) are practically negative NO-donors. In an intermediate position are the aliphatic N-nitrosamines of 7-azabicyclo[2.2.1]heptanes **13** and certain aromatic N-nitrosamines **14**. On the other hand, the same interesting study shows that, by analysing the ·NO released from compound **12** with a spin trapping reagent (the potassium salt of 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxy-3-oxide), this N-nitroso compound releases NO very quickly in acidic medium (pH 3.8) as a result of the homolytic cleavage of the N—NO bond, whereas *e.g.* bicyclic-N-nitrosamines release NO very slowly (~24 hr to completion) which is interpreted in terms of the formation of NO^+ ion upon acid-catalyzed heterolytic cleavage of the N—NO bond.

The photochemical and thermochemical decomposition of alkyl nitrites first leads to homolysis of the O-NO bond (the homolytic bond dissociation energies range between 150-170 kJ/mol) followed by either H-abstraction in the case of straight-chain nitrites or ring opening for cyclic nitrites [63-67]. Due to the high electronegativity of O, the N-atom exhibit high electron deficiency, rendering them highly susceptible to nucleophilic attack by common nucleophiles (amines, thiols, carbanions), leading to NO^+ transfer in the so called transnitrosation processes which are very often in solution. However, the transformation of nitrites to NO requires one-electron reduction: S-nitrosothioles may be formed from alkyl nitrites in vivo and because of that, some of them have been clinically used as vasodilators for a long time.

S-Nitrosothiols (thionitrites) decompose on heating, or in some cases on standing, by irradiation or in solution. The decomposition may involve homolytic and heterolytic cleavage of the S–NO bond. The thermal reaction involves the homolytic cleavage of the S–NO bond that leads to NO and sulfanyl radicals in a reversible reaction, which finally yield the corresponding disulfide as the unique reaction product, eq. **6**. The process is slow, for example the half-lives for the decomposition of S-nitrosocysteine or *tert*-butylthionitrite in solution at 25 ºC are ca. 55 h or 75 h respectively. The activation energies (ca. 85-96 kJ/mol) are significantly lower than the S–N dissociation bond energy [68].

$$RSNO \rightleftharpoons NO\cdot + RS\cdot \longrightarrow RSSR \qquad (6)$$

The photochemical cleavage of S–NO bond, *e.g.* upon irradiation of S-nitroso-glutathione **8** at the absorption bands at either 340 or 545 nm, results in the release of NO and thiyl radicals [69]. Decomposition of RSNOs in solution is accelerated by the presence of certain metal ions like Fe^{+2}, Hg^{+2}, Ag^+ but the catalytic effect is really important for the case of Cu^{+2} [70]. Since the human body contains around 100 mg of copper per 75 kg of body weight and it is widely distributed in the blood, bone and muscle, the catalytic effect of Cu^{+2} on RSNO decomposition is a topic of high interest. Williams' research group has devoted many efforts to the knowledge of this process, due in part to the irreproducible results obtained in kinetic studies in water [71, 72]. The reaction mechanism is now well-understood, and like it can be seen in Scheme **11**, the true catalyst is Cu^+, generated by thiolate reduction of Cu^{+2}. Reaction then occurs between Cu^+ and RSNO regenerating Cu^{+2} and RS^-, and releasing NO.

$$RSNO + H_2O \rightleftharpoons RS^- + NO_2^- + 2H^+$$

$$Cu^{+2} + RS^- \longrightarrow Cu^+ + {}^1/_2\,RSSR$$

$$Cu^+ + RSNO \longrightarrow Cu^{+2} + RS^- + NO$$

Scheme 11. Reaction mechanism of Cu^+ catalysis in RSNO release of NO.

Reduction of Cu^{+2} can also be promoted by ascorbate (the monoanion of ascorbic acid –vitamin C) at pH ≈7: at low ascorbate concentrations it acts as a reducing agent of Cu^{+2}, the major product being the disulfide RSSR; but at high ascorbate concentrations, it acts as a nucleophile, yielding electrophilic nitrosation to produce NO and the corresponding thiol [73].

3.3. Nitrosation in Acidic Medium

Nitrosating Agents

The most convenient and useful reagent for effecting nitrosation is nitrous acid, generated *in situ* in aqueous solution from sodium nitrite (henceforth nitrite) and mineral acid. The nitrous acid is a weak acid, whose pK_a at 25 ºC has been measured as 3.15 [74].

$$HNO_2 \overset{K_a}{\rightleftharpoons} NO_2^- + H^+ \qquad pK_a = 3.15 \tag{7}$$

In aqueous perchloric acid solutions of sodium nitrite the only nitrosating agent is NO^+ (or $H_2NO_2^+$, *i.e.* $ON^+\cdots OH_2$ where a discrete water molecule is covalently bound to the nitrosonium ion) which is formed from protonation of nitrous acid, eq. **8**

$$HNO_2 + H^+ \overset{K_1}{\rightleftharpoons} NO^+\cdot H_2O \qquad K_1 = 3.5\times10^{-7}\ M^{-1} \tag{8}$$

Depending on $[HNO_2]$ and on the medium acidity, the nitrous acid aqueous solutions can also generate dinitrogen trioxide N_2O_3, eq. **9**. High nitrous acid concentrations and low acidities favor the formation of N_2O_3 [75].

$$2\ HNO_2 \overset{K_2}{\rightleftharpoons} N_2O_3 + H_2O \quad K_2 = 3\times10^{-3}\ M^{-1} \tag{9}$$

In aqueous solutions of nitrous acid containing halide ions (F^-, Cl^-, Br^-, I^-), pseudo-halides, such as SCN^-, $S_2O_3^{-2}$, or even thiourea, in general non-basic nucleophiles, a new kind of nitrosating agents are generated *in situ*, the nitrosyl halides, XNO. The equilibrium constants for the formation of nitrosyl halides have been determined, when possible, by Schmid [76] and Stedman [77]. The corresponding equilibrium process is stated in eq. **10**, the values of K_3 at 25 ºC increase as 1.14×10^{-3}, 0.052, 32, and 5000 M^{-2} on going from ClNO, BrNO, SCNNO to nitrosothiourea, respectively.

$$HNO_2 + H^+ + X^- \overset{K_3}{\rightleftharpoons} XNO + H_2O \tag{10}$$

Rate Equations in Nitrosation

In strong mineral acid (ca. $[H^+]$>10 mM) and at low nitrite concentration (ca. [nitrite]<1 mM), the only detected nitrosating agent is the NO^+. The experimental rate equation is first-order in each nitrous acid, substrate (S), and free hydrogen ion concentration. Kinetic data obtained in the nitrosation of amines, amides, phenols, ketones, nitro compounds, carbanions, etc, are explained by means of eq. **11**. Values of k_1 ($=k_{NO}K_1$, with k_{NO} being the bimolecular rate constant of $S+NO^+$) lie in the range of 1-6000 $mol^{-2}dm^6s^{-1}$ at 25 ºC in water (Table **3**); k_1 values higher than 2000 are accepted for an encounter controlled process.

$$\text{rate} = k_1[HNO_2][H^+][S] \tag{11}$$

When the nitrous acid concentration is high (ca. $>10^{-2}$ M) and the acidity of the medium is low (ca. pH>2), the nitrosation reaction *via* N_2O_3 is normally detected. The key experimental feature is an additional term in the rate equation which is second-order in nitrous acid concentration, eq. **12**. Depending on the experimental conditions, both terms can be kinetically observed or only one of them.

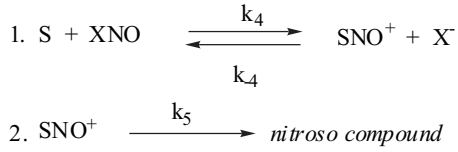
$$\text{rate} = k_1[HNO_2][H^+][S] + k_2[HNO_2]^2[S] \tag{12}$$

The intervention of nitrosyl halides XNO, X^-=Cl^-, Br^-, SCN^-, … (or pseudo-halides) in nitrosation reactions in aqueous medium can readily be deduced from the catalytic effect of halide ions. For instance, under experimental conditions of strong mineral acid and low nitrite concentrations, typical rate equations such as eq. **13** are often observed.

$$\text{rate} = (k_1 + k_3[X^-])[HNO_2][H^+][S] \tag{13}$$

The normal trend of reactivity observed in water for the several nitrosating agents is $NO^+ > NOCl > NOBr > NOSCN \approx N_2O_3 > NOTU$ (with NOTU symbolizing the nitrosothiourea cation). Table 3 displays values of the rate constants for the reaction between these nitrosating agents and different substrates [78-85]. The observed catalysis by X^- is due to the higher values of K_3 (*vide supra*) in comparison with K_1 (eq. **8**) or K_2 (eq.**9**), which means that the XNO concentration is much higher than that of either NO^+ or N_2O_3. This fact is clearly seen in the nitrosation of malononitrile in aqueous solutions of acetic acid [86]. The reactive species is the carbanion of malononitrile, $(CN)_2CH^-$, which is in minor proportions (pK_a=11.4) under the reaction conditions employed (2.5<pH<5); no nitrosation by NO^+, N_2O_3, or even NOCl is kinetically detected, but NOBr, NOSCN, or NOTU react with this carbanion with the same rate constant (equal to 1.1×10^{10}, 4.2×10^9, and 5.0×10^9 $mol^{-1}dm^3s^{-1}$, respectively), even though the observed catalysis increases in the order NOBr<NOSCN<NOTU. This finding makes the carbanion of malononitrile like the most reactive substrate studied in nitrosation.

On the other hand, depending on the nature of the substrate being nitrosated, three possible limit situations can be observed in reference to the general reaction mechanism of Scheme **12**: *(i)*if $k_5 >> k_{-4}[X^-]$, then the nitrosation reaction is rate-determining, step of rate constant k_4; *(ii)*if $k_5 << k_{-4}[X^-]$, the reaction of the nitroso intermediate, SNO^+, is rate-determining, in other works, the reversible nitrosation step is an equilibrium one, and *(iii)*when $k_5 \cong k_{-4}[X^-]$, situation of steady-state for the nitroso intermediate, SNO^+.



Scheme 12. General reaction mechanism for nitrosation in aqueous acid medium.

The first situation is typical for aliphatic secondary amines, in which case the reactivity of the different nitrosating agents towards the same substrate can be

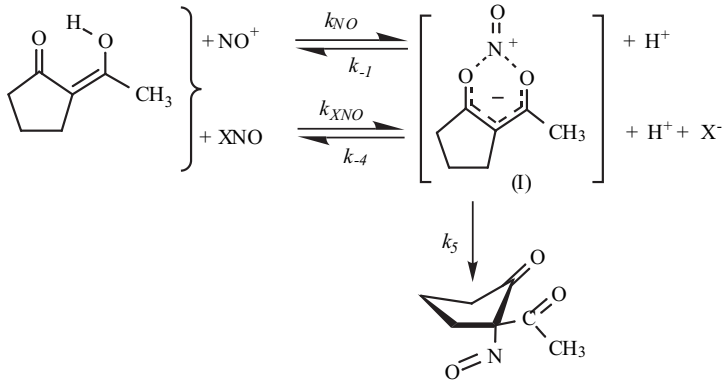
Table 3. Rate Constants Obtained in the Nitrosation of Some Substrates in Water at 25 ºC by the Nitrosating Agents NO^+ (k_1, eq 11), N_2O_3 (k_2, eq 12), and XNO {k_{XNO}= (k_3/K_{XNO}) with X^-=Cl^-, Br^-, SCN^-}

Substrate	pK_a	$k_1/M^{-2}s^{-1}$	$k_2/M^{-2}s^{-1}$	$k_{ClNO}/M^{-1}s^{-1}$	$k_{BrNO}/M^{-1}s^{-1}$	$k_{CNSNO}/M^{-1}s^{-1}$	ref.
aniline	4.60	2.0×10^4	2.5×10^6	3.0×10^9	2.7×10^9	1.9×10^8	76,78
1-naphthylamine	3.80	6400	---	2.2×10^9	3.7×10^9	3.8×10^9	79
morpholine	8.70	---	6.6×10^5	---	6.2×10^7	---	80
piperazine	5.55	1930	4.3×10^5	---	---	---	80
dimethylamine	10.87	7800	4.2×10^5	3.1×10^7	3.6×10^7	6.0×10^6	80
proline	10.6	2100	4.6×10^5	1.67×10^7	---	---	81
2-naphthol	8.8	4700	800	5.5×10^7	3.6×10^6	1.4×10^4	82
benzoylacetone	8.70	18.0	---	1.9×10^4	5.1×10^3	800	83
acetylacetone	9.08	14.5	---	7.2×10^4	3.3×10^3	730	83
TTeA [(a)]	6.16	2.6×10^4	2.7×10^5	8.8×10^8	6.6×10^8	---	84
2-nitropropane	5.10	3650	---	8.8×10^7	5.1×10^6	8.6×10^3	85

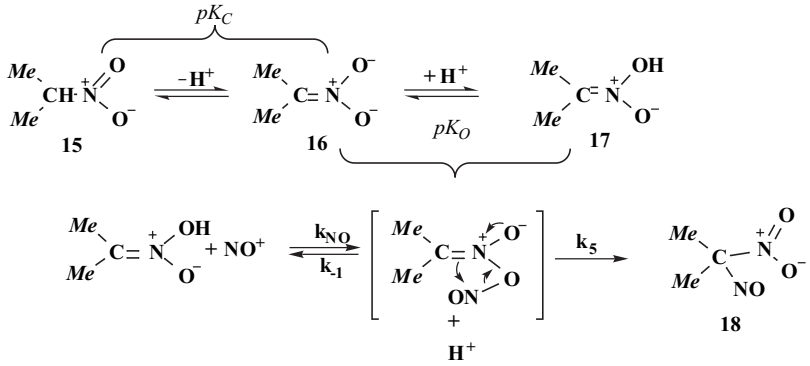
[(a)]Enol of 1,1,1-trifluoro-3-(2-thenoyl)acetone.

analysed. Nevertheless, in the nitrosation of amides –and their derivatives, ureas, carbamates, …– no catalysis by X^- is observed; in contrast, the reaction is subject to primary solvent isotope effects and to general base catalysis with bases of pK_a in the range 0.6-4.6. Both these features are indicative of a slow proton transfer from the nitroso intermediate to the base (the step of rate constant k_5 is rate-determining) [87]. Finally, as an example of the third situation it can be considered the nitrosation of the enol of 2-acetyl-cyclopentanone in strong aqueous acid medium [88]. Under pseudo-first order conditions with the concentration of 2-acetyl-cyclopentanone (ACPE) much lower than [nitrite], the rate equation shows good first-order dependence in the concentrations of both reagents, diketone and nitrite, but the dependence upon acidity is not linear, it has a downward curvature that is more pronounced when ClH is used instead $HClO_4$ and also the observed rate constant (k_o) does not increase linearly with the concentration of X^- (=Cl^-, Br^-, SCN^-), *i.e.* there is a tendency for the reaction to lose its first-order dependence on both [H^+] and [X^-] at high concentration levels. These kinetic features can be explained if the reaction mechanism outlined in Scheme **13** is assumed. The initial nitrosation reaction gives the *chelate-nitrosyl complex* intermediate that is in *steady-state*, which then undergoes an internal rearrangement to give the final C-nitroso compound. This proposal is supported by the fact that 1,3-diketones are used as chelating extractants of metal ions such as Fe^{+3}, In^{+3}, now well-documented in the literature [89].

The same pattern of behavior have been found in the nitrosation of aliphatic nitro compound **15** also in strong mineral acid, but in this case the reactant in excess was the nitro compound [85]. The postulated intermediate is the O-nitroso-nitrocompound formed in the nitrosation of the nitronic acid **17**. The O-nitroso intermediate then rearranges in the rate-determining step to the most stable *nitrolic acid* (primary nitro compound) or *pseudonitrole* **18** (secondary nitro compound), Scheme **14**. The nitronate anion **16** is generated from the aqueous alkaline solutions of nitro compounds and acidification of an alkaline solution of a nitro compound gives the nitronic acid which is stable enough relative to the nitro compound to allow nitrosation to occur. In order to observed the nitrosation reaction, the



Scheme 13. Postulated reaction mechanism of the nitrosation of ACPE in aqueous acid media.



Scheme 14. Nitrosation of 2- nitropropane in aqueous acid medium.

two stock solutions –the nitronate ion in aqueous alkaline medium and the sodium nitrite in aqueous strong acid medium– are mixed together, *e.g.* in the reaction cell of a stopped-flow apparatus.

Surprisingly, the nitrosation of other diketones such as acetylacetone, benzoylacetone [83] or ethyl 2-cyclohexanone carboxylate [90] shows the 'normal' pattern of behavior expected in nitrosation of ketones, *i.e.* linear dependences of k_o on both $[H^+]$ and $[X^-]$, in other words, the nitrosation is the rate determining step.

3.4. Nitrosation in Basic Medium

The basic solutions of nitrous acid content the NO_2^- ion. Neither nitrous acid nor nitrite ion cause nitrosation. The most studied nitrosating agents for the basic medium include alkyl nitrites, certain nitrosamines such as N-methyl-N-nitroso-*p*-toluenesulfonamide (MNTS, **5** in Scheme **8**), metal nitrosyl complexes such as sodium nitroprusside and thionitrites.

The nitrosation reaction takes place by the direct transfer of the NO-group from the NO-donor to the NO-acceptor in the so called *trans-nitrosation reaction*. Therefore, the rate equation is first order in both NO-donor (R-NO) and NO-acceptor (S) concentration, and independent of $[H^+]$ if the pH of the reaction medium is much higher than the pK_a of SH^+, eq. **14**.

$$\text{rate} = k_{tn}[\text{R-NO}][\text{S}] \qquad (14)$$

The above mentioned NO-donors are electrophiles that may undergo reactions with nucleophiles such as anions (*e.g.* OH^-) or amines (*e.g.* $(CH_3CH_2)_2NH$). The interaction between nucleophiles and electrophiles is expressed by two terms, an electrostatic and an orbital interaction term. When the reaction is *charge-controlled*, the electrostatic term dominates over the other; in this case a large energy gap separates the highest occupied molecular orbital (HOMO) of a nucleophile from the lowest unoccupied molecular orbital (LUMO) of an electrophile. The contrary situation corresponds to an *orbital-controlled* reaction, that is when the HOMO of a nucleophile and the LUMO of the electrophile are near in energy. According to the Pearson's principle of hard and soft acids and bases (HSAB), the interactions between hard nucleophiles-hard electrophiles (or soft nucleophiles-soft electrophiles) are expected to be stronger than the other possible combinations. The OH^- is the prototype of hard nucleophiles that fovor the charged controlled reactions; by contrast, amines are soft nucleophiles, then they favor orbital controlled reactions. In the same sense, the electronegativity difference between N and O is small, consequently the nitroso group bound to N-, O-, S- atoms (the situation of typical NO-donors) are low polarized, and thus it is considered a soft electrophile which reacts faster with soft nucleophiles than with hard ones. Therefore, one may expect faster reactions between the NO-donors and amines than with OH^-.

The reaction of alkyl nitrites (RO-NO) with OH^-, *i.e.* their *basic hydrolysis* to give the corresponding alcohol ROH and NO_2^-, has been largely studied [91, 92]. The process is very slow (the half live in 0.2 M NaOH ranges from 1h, in the case of activated alkyl nitrites such as 2-ethoxyethyl nitrite, to approximately 15h for the case of non-activated alkyl nitrites like cyclohexylnitrite. Electron-withdrawing substituents accelerate the reaction due to the higher stability of the alkoxide leaving group. The process in water is slower than *e.g.* the basic hydrolysis of carboxylic esters and is catalyzed by trifluoroethoxide anion (TFE). Rate enhancements by more than 3-fold are determined in the presence of trifluoroethanol in strong alkaline medium: the pseudo-first order rate constant increases in proportion to both OH^- and $F_3CCH_2O^-$ concentrations according to eq. **15**. Values of the bimolecular rate constants are reported in Table **4**.

$$k_o = k_{OH}[OH^-] + k_{TFE}[TFE^-] \qquad (15)$$

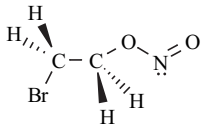
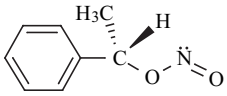
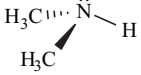
The *trans-nitrosation reaction* of amines by alkyl nitrites in alkaline (or basic) medium has been widely study [93, 94]. The observed second-order rate constant of NO transfer is unaffected by ionic strength of medium and decreases when the $[H^+]$ increases. These characteristics are typical of a reaction mechanism is which the slow step is the reaction between the alkyl nitrite and the non-protonated form of the amine, k_{tn}. Representative values of k_{tn}, *i.e.* the bimolecular reaction between RONO and the neutral amine, are reported in Table **5**.

It can be seen that, for a given amine like dimethylamine, the rate of the reaction is faster with the alkyl nitrites bearing electron-withdrawing substituents due to the higher stability of the leaving alkoxide (RO^-), in which case the –O-N- bond is partially broken early to the transition state in a mechanism more resembled to an S_N1 process. For a given alkyl nitrite, the rate of the nitrosation reaction increases with the basicity of the amine; however, if all available data are taken together, there is not a good

Table 4. Bimolecular Rate Constants for the Basic Hydrolysis by OH^- (k_{OH}) or by the Anion TFE (k_{TFE}) of Some Alkyl Nitrites and Esters at 25 ºC [91,92]

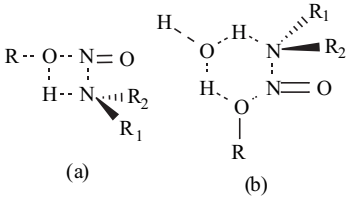
Substrate (S)	k_{OH}/ $mol^{-1}dm^3s^{-1}$	k_{TFE}/ $mol^{-1}dm^3s^{-1}$	medium
2-bromoethyl nitrite	0.010	---	water
2-ethoxyethyl nitrite	0.95×10^{-3}	3.2×10^{-3}	water
n-butyl nitrite	0.75×10^{-4}	0.23×10^{-3}	water
n-pentyl nitrite	1.0×10^{-4}	0.26×10^{-3}	water
cyclohexyl nitrite	0.6×10^{-4}	---	water
1-phenylethyl nitrite	5.2×10^{-4}	---	water
2-phenylethyl nitrite	3.3×10^{-4}	---	water
1-phenyl-1-propyl nitrite	4.7×10^{-4}	0.81×10^{-3}	water
3-phenyl-1-propyl nitrite	2.2×10^{-4}	0.53×10^{-3}	water
n-butyl nitrite	0.6×10^{-4}	---	61v% dioxane-water 35 ºC
1-phenylethyl nitrite	1.2×10^{-4}	---	61v% dioxane-water 35 ºC
cyclopentylbenzoate	1.2×10^{-3}	---	61v% dioxane-water 35 ºC
1-phenylethylbenzoate	2.1×10^{-3}	---	61v% dioxane-water 35 ºC
2-phenylethylbenzoate	7.8×10^{-3}	---	61v% dioxane-water 35 ºC
Benzylbenzoate	6.8×10^{-3}	---	61v% dioxane-water 35 ºC
n-propylformate	41.2	---	water

Table 5. Bimolecular Rate Constants for the Nitrosation of Amines by the Alkyl Nitrites, 2-bromoethyl Nitrite and 1-Phenylethyl Nitrite, and of Dimethylamine with Various Alkyl Nitrites, Performed in Water at 25 ºC

amine				
	k_{tn}/$mol^{-1}dm^3s^{-1}$	k_{tn}/$mol^{-1}dm^3s^{-1}$	alkyl nitrite	k_{tn}/$mol^{-1}dm^3s^{-1}$
methylethylamine	2.40	0.22	CH_3-ONO	0.094
diethylamine	1.01	0.074	CH_3CH_2ONO	0.064
methyl-*iso*-propylamine	1.02	0.093	$OH(CH_2)_2$-ONO	0.503
methylbutylamine	2.90	0.302	$EtO(CH_2)_2$-ONO	0.66
dibutylamine	1.20	0.108	$ClCH_2CH_2$-ONO	3.42
N-methylcyclohexylamine	1.56	0.140	CF_3CH_2-ONO	718
Piperidine	2.70	0.227	$CH_3(CH_2)_2$-ONO	0.066
2-methylpiperidine	0.44	0.043	$(CH_3)_2CH$-ONO	0.022
4-methylpiperidine	2.20	0.20	$(CH_3)_3C$-ONO	0.0087
pyrrolidine	14.1	1.60	---	---

correlation between $log(k_{tn})$ and the pK_a of amines, nevertheless, good relationship is found if, for example, only the series of aliphatic cyclic-amines (pyrrolidine, piperidine, piperazine, morpholine, …) is considered [93]. Another important characteristic of the nitrosation reaction is its large and negative values of the activation entropy, which suggest that the reaction takes place *via* a highly ordered

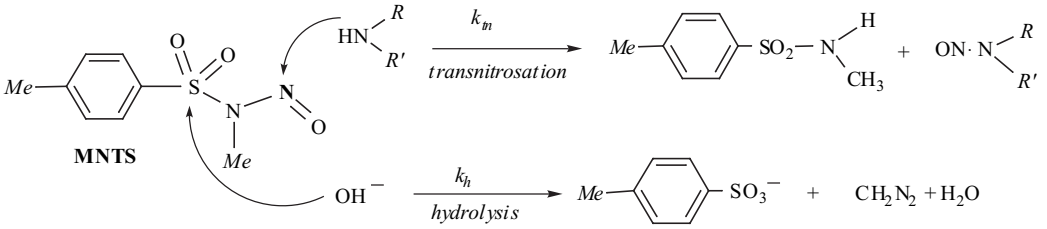
transition state. A cyclic-structure of four centres in which the amine aids removal of the –OR group by partial protonation, of even of six-centres that includes also a water molecule, (Scheme **15**) have been postulated to explain the entropy values.



Scheme 15. Possible structures for the transition-state in the NO-transfer from RONO to amines in water.

MNTS is an ambident electrophile, *i.e.* the N of the nitroso group and the S are electrophilic centers. Both centers discriminate between different nucleophiles; for example, OH^-, ClO^- or EtO^- attack on the S-atom to afford diazometane and toluene-*p*-sulfonate, whereas the reaction with amines, enolates, nitronates, … goes through the NO-group, Scheme **16**.

The rate of NO-transfer from MNTS to different nitrogen nucleophiles including primary, secondary and tertiary amines, as well as, N_3^-, H_2NNH_2, NH_3, ..., has been studied in water at 25 ºC [95]. Secondary amines produce stable N-nitrosamines in quantitative yield. The N-nitrosamines formed from tertiary amines decompose in basic medium by generating NO_2^- and the recovery of the



Scheme 16. Nucleophilic reactions of MNTS in aqueous alkaline medium.

Table 6. Bimolecular Rate Constants for NO-Transfer from N-Nitroso-*p*-Toluenesulfonamide to Primary, Secondary, and Tertiary Amines in Water at 25 ºC

Nº	Amine	pK_a	$k_{tn}/M^{-1}s^{-1}$	Nº	Amine	pK_a	$k_{tn}/M^{-1}s^{-1}$
Primary amines				**20**	4-hydroxyproline	9.66	7.52×10^{-2}
1	glycylglycine	8.25	5.30×10^{-5}	**21**	N-methylbenzylamine	9.71	4.10×10^{-2}
2	methionine	9.28	1.51×10^{-4}	**22**	piperazine	9.73	2.98×10^{-2}
3	2-methoxyethylamine	9.43	9.81×10^{-4}	**23**	sarcosine	10.2	4.87×10^{-2}
4	ethanolamine	9.50	7.70×10^{-4}	**24**	N,N'-dimethylethylenediamine	10.29	0.141
5	glycine	9.78	8.94×10^{-4}	**25**	proline	10.64	0.129
6	2-aminobutyric acid	9.83	3.24×10^{-4}	**26**	dimethylamine	10.77	0.384
7	ethylenediamine	9.93	5.39×10^{-3}	**27**	diethylamine	10.93	5.81×10^{-2}
8	*iso*-butylamine	10.48	7.06×10^{-3}	**28**	dipropylamine	11.00	6.32×10^{-2}
9	*sec*-butylamine	10.56	1.34×10^{-3}	**29**	piperidine	11.12	0.16
10	propylamine	10.57	9.16×10^{-3}	**30**	diisopropylamine	11.20	1.12×10^{-2}
11	butylamine	10.64	5.46×10^{-3}	**31**	pyrrolidine	11.30	0.83
12	ethylamine	10.64	6.78×10^{-3}	*Tertiary amines*			
13	methylamine	10.64	1.80×10^{-2}	**32**	1,4-diazobicyclo[2.2.2]octane	8.82	2.87×10^{-2}
14	*iso*-propylamine	10.67	1.40×10^{-3}	**33**	trimethylamine	9.80	0.24
Secondary amines				**34**	quinuclidinol	9.86	4.90×10^{-2}
15	thioproline	6.20	2.33×10^{-4}	**35**	N-methylpiperidine	10.08	2.80×10^{-2}
16	sarcosine ethyl ester	8.12	4.82×10^{-3}	**36**	diethylmethylamine	10.54	5.08×10^{-2}
17	morpholine	8.49	4.96×10^{-3}	**37**	triethylamine	10.71	1.84×10^{-2}
18	N-methylpiperazine	8.98	7.81×10^{-3}	**38**	quinuclidine	11.15	0.44
19	thiomorpholine	9.06	1.23×10^{-2}				

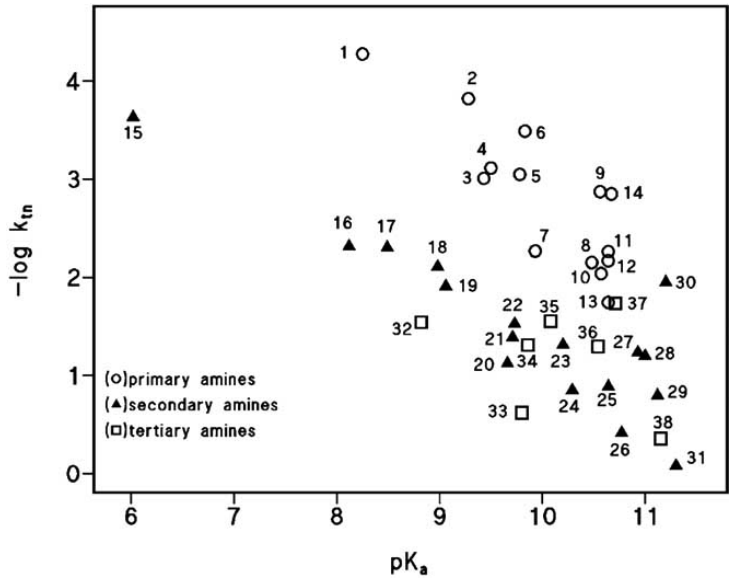
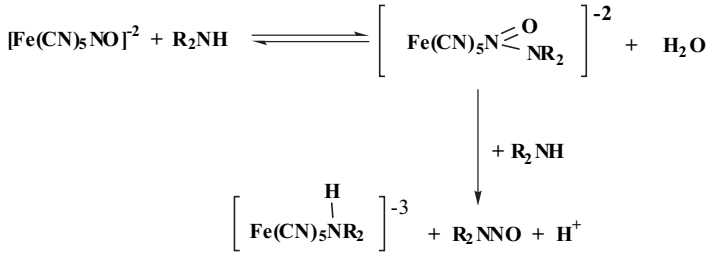


Fig. (3). Bronsted plot for the NO-transfer reaction from N-nitroso-toluene-p-sulfonamide to amines.



Scheme 17. Mechanism of reaction of nitroprusside ion with secondary amines in basic medium.

amine; even though alternative pathways for the decomposition are possible, this *via* represents more than 90% based on the NO_2^- generated. Primary amines react with MNTS considerable more slowly than secondary or tertiary amines of similar basicity, Table **6**. However, taken the data all together, no correlation or clear trend are shown as it can be seen in Fig. **3**. Poor correlations are also obtained if the vertical ionisation potential of the nucleophile is used instead of basicity. Nevertheless the empirical reactivity parameter, the Ritchie's N^+ index [96], seems to correlate all together the reactivities of primary, secondary and tertiary amines with MNTS. Possible reasons to explain this feature can be found if one admits that the index N^+ measures some property of the nucleophiles that strongly influences reactivity. This assumption is supported by the observation that reactivities of MNTS correlate quite well with the reactivities towards the same nucleophiles of different alkyl nitrites measured in different conditions such as 61% dioxane-water mixtures.

The nitroprusside ion, $[Fe(CN)_5NO]^{-2}$ (NP), acts also as a NO-donor in neutral or weakly basic medium. In recent years, research in the modes of interactions of NO-donors with metalloporphyrins has attracted considerable interest, since the binding modes should determine the chemical reactivities of such groups [97]. Linear nitrosyl complexes such as NP (see Scheme **6**), in which NO is formally considered to exist as NO^+, react with nucleophiles like secondary amines to give nitrosamines.

Under experimental conditions of pH<9, the rate equation is first-order on [NP] and second-order on [amine]. The second order on amine is explained by a mechanism of two steps: the first one is a fast reaction between the amine and the coordinated nitrosyl group to form an unstable complex in equilibrium with the reagents, and then reacts with another amine molecule to produce the corresponding nitrosamine and the complex $[Fe(CN)_5(amine)]^{-3}$, Scheme **17**.

In strong basic medium (pH>11) the nitrosyl complex converses completely to the nitro complex, which in the presence of amines, an amine molecule may replace the NO_2^- ion, Scheme **18**.

4. EFFECT OF CYCLODEXTRINS ON NITROSATION REACTIONS

As we have already pointed out, the most remarkable property of cyclodextrins is their ability to form inclusion compounds that exist even in aqueous solutions. This unique feature of CDs might induce significant modifications in the *position equilibrium processes*, by the preferable inclusion of one component over the other –the Le Châtelier principle– or *enhance/decrease the reactivity* of a substrate, mainly due to changes in its conformation upon inclusion, to its encapsulation that protects it from another reagent in a bimolecular reaction, or due to the promotion of

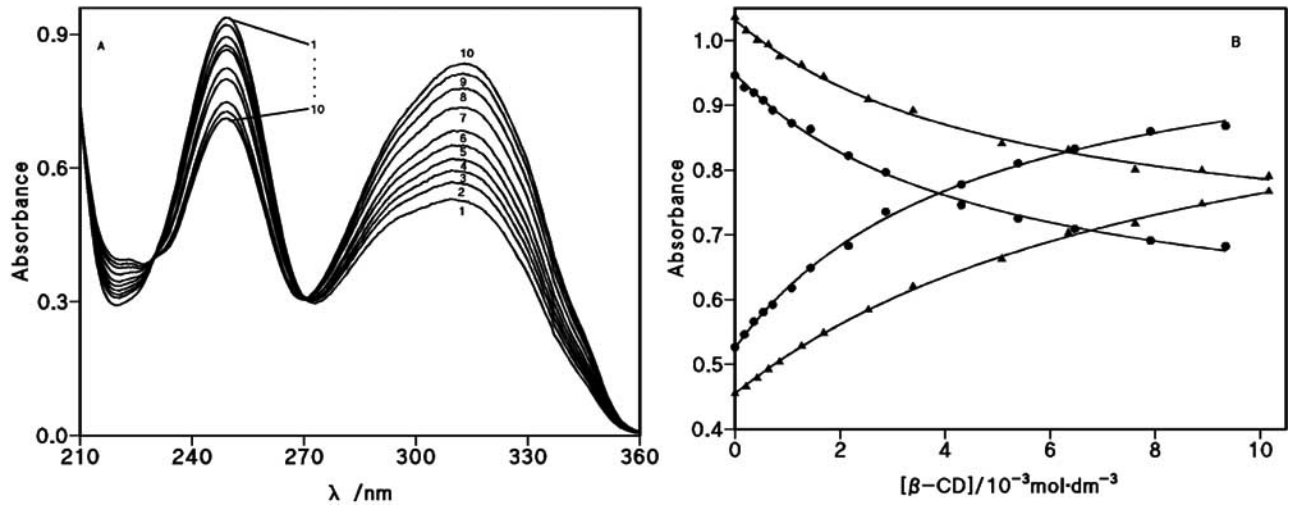
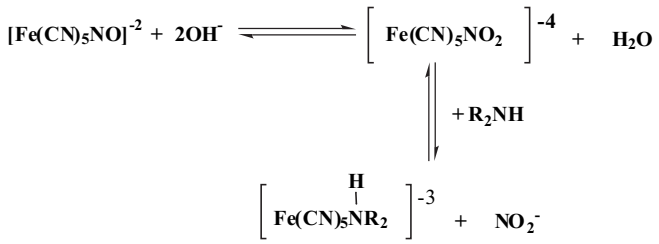


Fig. (4). (a) Absorption spectra of BZA (7.0×10^{-5} mol dm^{-3}) in the presence of [HCl]= 0.033 mol dm^{-3} as a function of β-CD concentration at: **(1)** 0.0; **(2)** 0.36; **(3)** 0.72; **(4)** 1.08; **(5)** 1.44; **(6)** 2.16; **(7)** 2.89; **(8)** 4.31; **(9)** 5.39; **(10)** 6.47 *m*mol dm^{-3}; (b) influence of β-CD concentration on the absorbance readings at 312 nm (ascending curves) and 250 nm (descending curves) at (•)10 °C and at (▲) 36 °C .

the reaction in a covalent interaction with the substrate, *i.e.* the CD behaves as an artificial enzyme.



Scheme 18. Conversion of the nitrosyl complex in the nitro complex in strong basic media.

This section details with the effect of β-CD on the hydrolysis reaction of nitroso compounds, either in aqueous acid or basic medium, on the formation of nitroso compounds both in acid and basic medium, and on the equilibrium position of nitrosatable substrates like ketones.

4.1. Modification of β-Cyclodextrin of Keto-enol Equilibrium

1,3-Diketones such as benzoylacetone (1-phenyl-1,3-butadione, BZA) exist is aqueous non-basic medium in equilibrium with their keto and enol tautomers. This type of equilibrium is very sensitive to the solvent nature, in a way that non-polar and/or aprotic solvent like cyclohexane strongly increase the amount of the enol, whereas polar and/or protic solvents stabilize the keto isomer. In solution the enol tautomer exists in a cyclic form stabilized by intramolecular hydrogen bonding, consequently the competition of intermolecular hydrogen bonding with the solvent depresses the enol amount.

The UV-vis absorption spectrum of BZA dissolved in water exhibits strong absorption at 250 nm and weak absorption at 312 nm [98]. On the contrary, in cyclohexane solvent the strong absorption is observed at 312 nm (λ_{max}=306 nm) whilst the weak band appears at 250 nm (λ_{max}=245 nm). The absorption band near to 312 nm is due to the enol tautomer, whereas that at 250 nm is due to the keto tautomer. Titration of an aqueous solution of BZA with β-CD produces a strong increases of the absorption intensity of the band centered at 312 nm with the concomitant decrease of the absorption at 250 nm, Fig. **4A** [99]. This is due to the formation of inclusion complexes with the enol tautomer, preferentially.

According to Scheme **19** an equilibrium exists in water between the keto and enol tautomers, K_E, as well as between both free keto and free enol and their 1:1 inclusion complexes with β-CD, which are characterized by the binding constants K_c^K and K_c^E, respectively. By assuming the value of K_E=0.62 determined for this fast equilibrium in water, the quantitative treatment for the absorbance increase at 312 nm (or for the absorbance decrease at 250 nm), Fig. **4B**, as a function of β-CD concentration, the values of the inclusion constants reported in Table **7** have been obtained. The increase in temperature destabilizes the inclusion complexes.

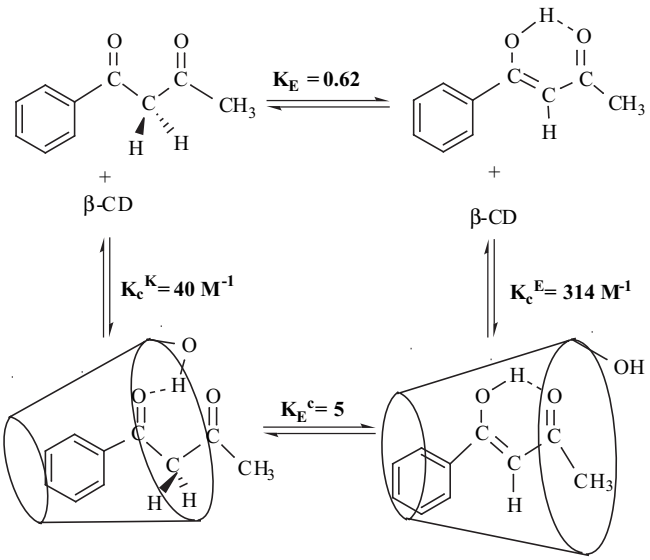
The optimized geometries of both tautomers indicate that the only way the planar enol molecule or the nonplanar keto molecule can fit into the β-CD cavity is lengthwise, but the encapsulation cannot be complete: the enol is deeper inside the β-CD cavity than the keto tautomer, which could be H-bonded to the secondary OH-groups of the wider rim, a fact that forces the keto isomer to be located more outside the cavity. The enthalpy and entropy values for the inclusion process listed in table **7** agree with such a picture for these complexes: the H-bonding interactions of the keto isomer are responsible of the higher enthalpy variation; moreover, the higher negative entropy value for the inclusion of the keto isomer is the consequence of higher number of water molecules that interact with this form in the bulk water solvent and remain free in the inclusion process. Additional insights about the complex geometries are obtained from ^{1}H NMR results: the CH_3-signal for the keto tautomer (δ=2.1

Table 7. Equilibrium Constants Corresponding to the Inclusion Complexes Formed between either Enol (K_c^E) or Keto (K_c^K) Tautomers of Some Diketones and β-CD in Water along with the Thermodynamic Parameters of the Inclusion Process

Diketone	λ_{max} (enol)/nm	T / °C	K_c^E/ M^{-1}	K_c^K/ M^{-1}	ΔH^E (kJ/mol)	ΔS^E (J/mol·K)	ΔH^K (kJ/mol)	ΔS^K (J/mol·K)
BZA	312	10.0	431	81.1	enol inclusion		keto inclusion	
	312	15.3	387	61.4				
	312	20.3	348	46.7				
	312	25.0	314	40.2	–14.7	–1.4	–33.7	–82.5
	312	31.1	283	28.7				
	312	36.0	254	24.0				
DBM	345	25	445	163	---	---	---	---
AcAc	245	25	*No complex detected*		---	---	---	---
ACHE	291	25	95	*No complex*	---	---	---	---

ppm in D_2O) does not shift by the presence of β-CD, contrarily to that observed for the same signal corresponding to the enol tautomer (δ=2.0 ppm) that both shift to higher δ-values and higher area in the presence of increasing amounts of β-CD.

The perturbation of the UV-vis absorption spectra of dibenzoylmetane (DBM) by CD addition has also been study to determine the equilibrium inclusion constant of this substrate. In the same manner we have observed that the presence of β-CD has no appreciable effects on the absorption spectrum of acetylacetone (AcAc) (neither on the observed rate constant of nitrosation in acid medium), because AcAc is quite soluble in water and no inclusion complex appears to form with β-CD. In the case of 2-acetyl-cyclohexanone (ACHE) system the enolization is a slow process that is retarded in the presence of CDs and the position of the keto-enol equilibrium is shifted to the enol side in aqueous β-CD solutions due to the formation of 1:1



Scheme 19. Keto-enol equilibria of BZA in aqueous β-CD solutions; values of equilibrium constants refer to 25 ºC.

inclusion complexes between the enol and the CD. The quantitative treatment of these spectroscopic changes affords the K_c^E value in Table **7** [100].

4.2. Effect of Cyclodextrins on Reactivity in Aqueous Acidic Medium

Another common way to experimentally determine the equilibrium constants for inclusion complexation is found in the analysis of the effect of CDs on reactivity. The reactivity of the included guest changes with respect to that of free guest: the total or partial encapsulation might protect a substrate from further reactions.

In aqueous acid or neutral medium, none –OH groups of CDs is ionized, *i.e.* the host is a neutral molecule whose cavity is filled of water molecules, called by Saenger [101] as *"activated water"*, therefore, inclusion of a hydrophobic substrate is preceded by the release of these high-enthalpy water molecules that makes unpredictable the balance of the driving forces of inclusion complex formation [102]. It has been frequently observed that small cations such as H^+, NH_4^+, protonated amines, or non-polarizable metal cations do not form inclusion complexes; the contrary occurs for anions. As a consequence, reactions that involve H^+, NO^+, …, such as acid hydrolysis, enolization, or nitrosation are good systems to analyse cyclodextrin-complexation processes with hydrophobic substrates. Next, we report some examples of these type of simple reactions.

Enol Nitrosation

As we have pointed elsewhere, in aqueous perchloric acid the only nitrosating agent at low nitrite concentrations is the NO^+ (or its hydrated form, NO^+OH_2, eq. **8**). In a reaction medium containing X^- (X^-=Cl^-, Br^-, SCN^-, …) nitrosation *via* the nitrosyl salt (XNO, eq. **10**) is also possible. On the other hand, the nitrosation of ketones goes only through the enol tautomer; therefore, this is a good reaction for studying the complexation of the enol by cylodextrins.

The cyclic structure of the enol of 1,3-dicarbonyl compounds stabilized by intramolecular hydrogen bonds, increases the hydrophobic character of this tautomer whose

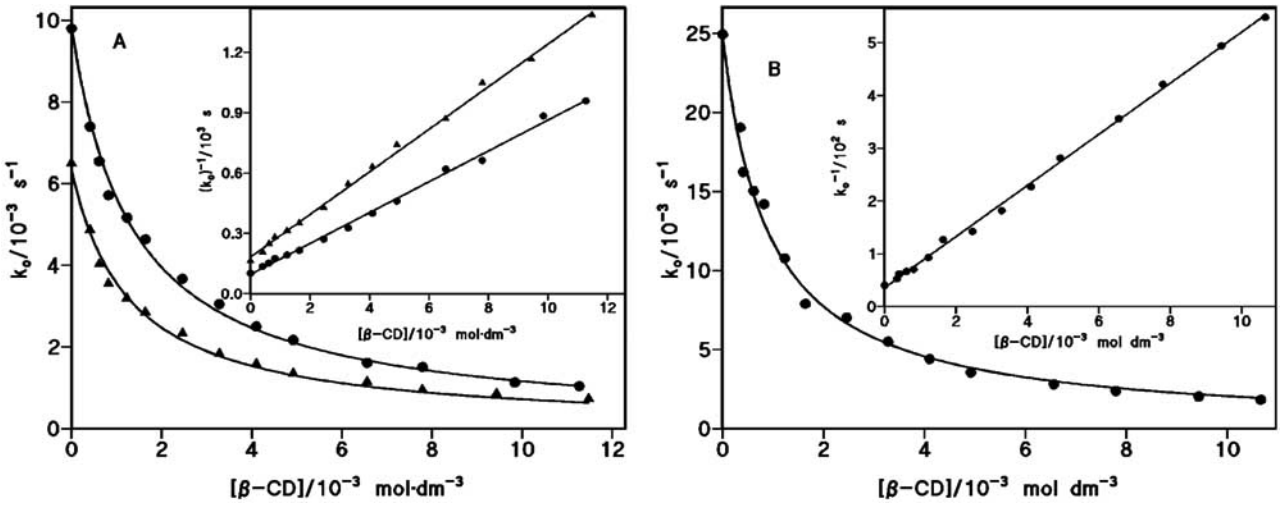
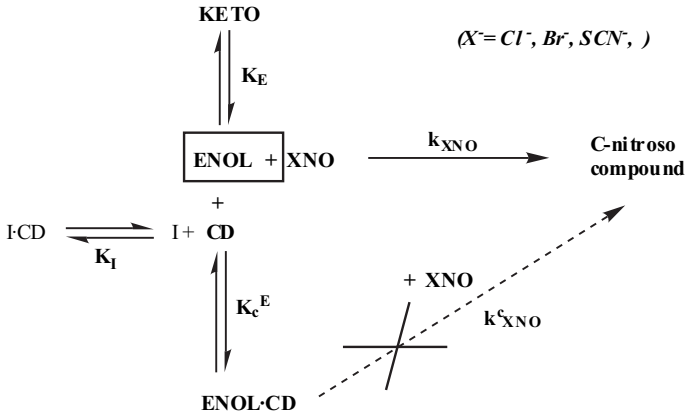


Fig. (5). Variation of k_o as a function of β-CD concentration for the nitrosation of the enol of ethyl-2-cyclohexanone carboxylate in aqueous acid medium at [nitrite]=1.67 mM and (A) at [$HClO_4$] equal to 0.017 M (triangles) and 0.025 M (circles), and (B) at [HCl]=0.050 M. The insets show the reciprocal plot of k_o against of β-CD concentration.

percentage is consequently enhanced in apolar and/or aprotic microenvironments. Therefore, the inclusion complexes formed between the enol and CDs protect the former from the attack by NO^+ (or XNO). Then, one must expect an *inhibition* of CDs in the nitrosation of 1,3-dicarbonyl compounds in aqueous acid medium, in accordance with the experimental observations (see the Fig. **5**) [90,99,100].

The reaction mechanism that explain the inhibition of CDs in the nitrosation of enols in aqueous acid medium is that of Scheme **20**, from which the eq. 16 can easily derived when the following conditions are fulfilled: *(i)*only 1:1 inclusion complexes are formed, *(ii)*the complexes are unreactive, *i.e.* k^c_{XNO}~0 or k^c_{XNO}<<k_{XNO}, and *(iii)*the host concentration is much higher than that of the guest, *i.e.* $[CD]_o$>> $[diketone]_t$, with $[CD]_o$ being the stoichiometric CD-concentration and k_W represents the observed rate constant in the absence of CDs.

The concurrence of the previous premises implies that the plot of $1/k_o$ against [β-CD] must be a straight line, whose slope is directly related to the value of K_c^E. On increasing the [$HClO_4$], the slope of the straight line decreases (inset of Fig. **5A**). As k_W depends on the [H^+] but not on the nature of the acid, the difference in the gradients of the straight lines could be due to K_c^E values, but this possibility is not understandable because the guest –the enol– remains the same, as in its conformational structure as in its nature. The origin of this apparent dilemma is the formation of inclusion complexes between β-CD and anions, such as ClO_4^-, Br^-, or AcO^- (acetate). Highly polarizable anions, like these, would act as *potential inhibitors*, I (even though the effect observed here is a catalysis because the complexes are unproductive), in the sense that these anions compete with the enol for the CD-cavity (the *active centre* in enzymology). Therefore, the introduction of the following equilibrium step: **β-CD + I** $\rightleftharpoons$ **β-CD·I, K_I** in Scheme **20** and taking now into account that $[\beta\text{-CD}]_o=[\beta\text{-CD}]+[\beta\text{-CD·I}]$, the new expression of eq. 17 results to relate the variation of k_o as a function of total β-CD concentration. From eq. 17 it can be noted the effect of [I] on the apparent values of K_c^E: $K_c^{app}= K_c^E/(1+K_I[I])$. Table **8** reports rate and equilibrium constants obtained in the nitrosation of diketones.



$$k_o = \frac{k_W}{1 + K_c^E [CD]_o} \qquad (16)$$

Scheme 20. Nitrosation in aqueous CD solutions.

Table 8. Rate and Equilibrium Constants Obtained in the Nitrosation of Enol of 1,3-Dicarbonyl Compounds in Aqueous Acid Medium in the Presence of β-Cyclodextrin at 25 ºC [90,99,100]

Substrate	Aqueous Medium (c, M)	$k_w/10^{-3}$ s^{-1}	K_c^E/M^{-1}	K_c^{app}/M^{-1}	K_I/M^{-1}
ECHC [a]	HCl (0.050)	24.9	1156	---	2.56
	$HClO_4$ (0.050)	17.8	1156	629	15.5
	$HClO_4$ (0.025)+$NaClO_4$ (0.085)	9.05	1156	477	13.4
	HBr (0.017)	19.25	929	---	5.6
BZA [b]	HCl (0.030)	0.396	314	---	---
	HBr (0.030)	0.95	314	---	---
ACHE [c]	HCl (0.033)	7.78	117	---	---
α-cyclodextrin			K_{11}/M^{-1} [d]	K_{12}/M^{-2} [e]	
ACHE	HCl (0.033)	8.05	20	247	---

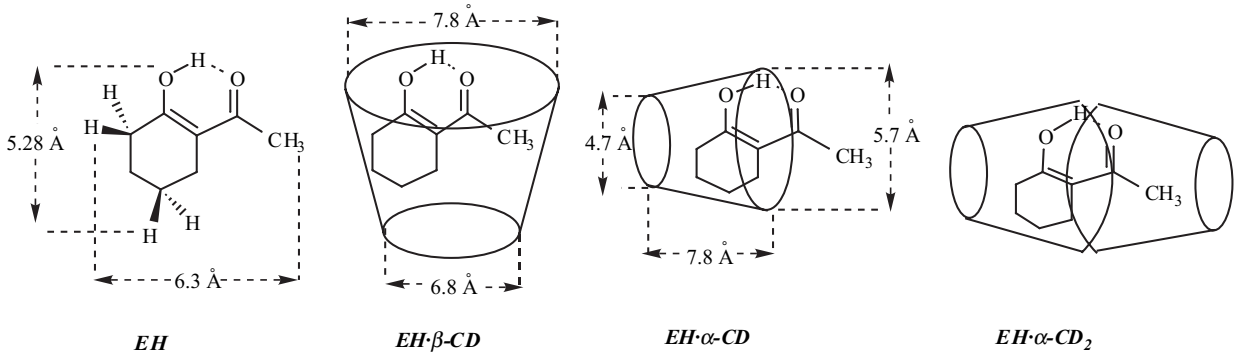
[a]ethyl 2-cyclohexanonecarboxylate; [b]benzoylacetone; [c]2-acetyl-cyclohexanone; [d]stability constant of the 1:1 inclusion complex formed between the enol and α-CD; [e]stability constant of the 1:2 inclusion complex formed between the enol and α-CD.

$$k_o = \frac{k_w}{1 + \frac{K_c^E}{1 + K_I[I]}[CD]_o} \quad (17)$$

In aqueous acid medium, ethyl cyclohexanone-2-carboxylate undergoes hydrolysis of the ester function. The reaction is more than 20-fold slower than the corresponding nitrosation reaction. The ester hydrolysis reaction is acid-base catalyzed and has been analysed under different experimental conditions. In every case, addition of β-CD inhibits the reaction by a factor of approximately 6 at the higher [β-CD] used, whose factor decreases in the presence of ClO_4^- or acetic acid/acetate [90].

The enol-ketonization reaction of 2-acetyl-cyclohexanone (ACHE) is a slow process which is accelerated by both acids and bases and is also inhibited by cyclodextrins. The degree of inhibition by β-CD is practically the same evaluated either in enol-nitrosation or in enol-ketonization reactions: a reduction in k_o of approximately 2-times is observed for both reactions. By contrast, the effect of reduction of k_o by α-CD addition is near double in nitrosation than in enol-ketonization reaction. Moreover, the reciprocal plot of k_o against [α-CD] is not linear. Figs. **6B** and **7B** show that an up-curve is drawn at high [α-CD]. These features are explained by considering the simultaneously formation of inclusion complexes between the enol of ACHE and α-CD of stoichiometries 1:1 and 1:2 (enol: α-CD). The stability of 1:2 inclusion complex is higher than that of 1:1 stoichiometry (compare values of K_{11} and K_{12} in Table 8), and it is kinetically observable only at high [α-CD] because it needs two α-CD molecules per complex. As two α-CD molecules are capable of totally encapsulating the enol tautomer, the 1:2 complex is unreactive towards both reaction processes, nitrosation and enol-ketonization, whereas the 1:1 complex showed to be reactive in enol-ketonization promoted by the solvent and this finding accounts for the high degree of inhibition found in nitrosation with respect to enol-ketonization [100].

The different behavior found with α-CD in relation to the experimental observations with β-CD can be understood by looking at the size and dimensions of the guest and the both hosts which leads to the proposal of the structures of Scheme **21**.



Scheme 21. Possible structures of the inclusion complexes formed between the enol (EH) of 2-acetylcyclohexanone and α-CD and β-CD.

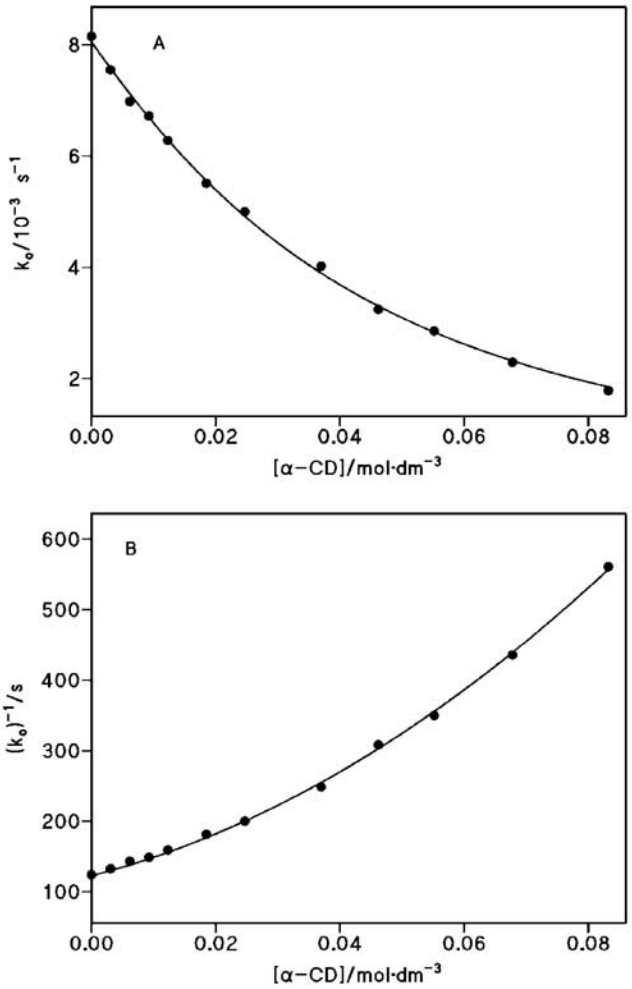


Fig. (6). (A) Plot of k_o against [α-CD] for the nitrosation of the enol of ACHE at [H^+]=0.033 M, HCl and [nitrite]=1.7 mM ; (B) reciprocal plot of k_o *versus* [α-CD].

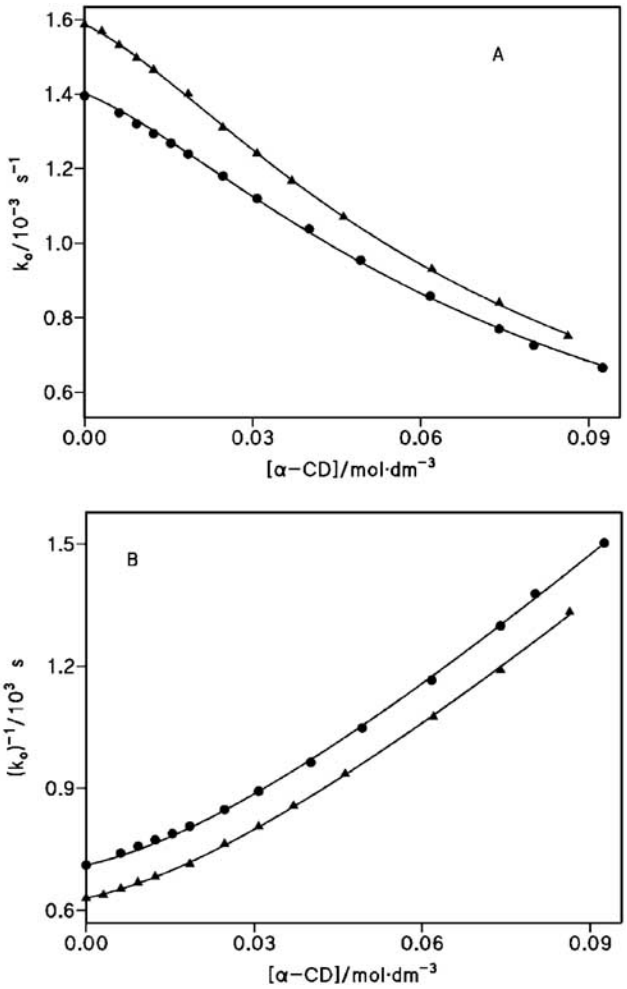


Fig. (7). (A) Plot of k_O against [α-CD] for the enol-ketonization reaction of ACHE at [H^+] equal to (•)0 and (▲)0.033 M, HCl; (B) reciprocal plot of k_o *versus* [α-CD].

Hydrolysis of Alkyl Nitrites

The effect of the molecular structure of the guest can be evaluated in the acid hydrolysis of aliphatic alkyl nitrites [103,104]. In contrast to the corresponding alcohol, alkyl nitrites are volatile compounds sparingly soluble in water. The study has been performed in both strong (HCl) and mild (pH~4.9, acetic acid/acetate buffer) acid media. In every case, addition of β-CD depresses the rate of acid hydrolysis as a consequence of the formation of unproductive 1:1 inclusion complexes between the alkyl nitrite RONO and β-CD (Fig. **8A**). That is, β-CD encapsulates the alkyl nitrites protecting them from the attack by both H^+ and acetic acid; in fact, addition of dodecyltrimethylammonium bromide (DTABr) at fixed β-CD concentration, increases again the observed rate constant (Fig. **8B**) to the level measured in water in the absence of CD. The resulting effect is due to the competition by the CD cavity of DTA cations, whose binding constant has been determined [105] as 3000 M^{-1}, a value much higher than that corresponding to any alkyl nitrite, see Table 9. This table depicts values of the observed rate constant in water, k_o, and in the presence of 0.010 M of β-CD, along with the stability constants of the inclusion complex, for several alkyl nitrites.

The structure of the RONO is of key importance in predicting the degree of inhibition that varies from 0 to 7, approximately. The lack of β-CD effect found in the case of 2-ethoxy-ethyl nitrite is explained by assuming the formation of an inclusion complex in which the ether-oxygen forms hydrogen bonds with the secondary −OH groups of the wide rim of the CD. This geometry of the complex forces the −ONO group to reside outside the CD cavity, which leaves immersed in the bulk aqueous medium. On the other hand, the maximum inhibition effect is found with the alkyl nitrites of compact structure, such as cyclohexyl- or *tert*-butyl nitrite, or when the NO-group is deeper inside the β-CD cavity, like in the case of 1-phenyl-1-propylnitrite. As it can be seen the higher stability of the inclusion complex (high K_c^N values) do not imply the higher degree of inhibition, in other words, the size and the structure of the guest are the decisive factors that control the effect of CD.

4.3. Effect of Cyclodextrins on Reactivity in Basic Media

The effect of β-CD on the basic hydrolysis of alkyl nitrites is an example of covalent catalysis. In aqueous alkaline medium (of ca. [OH^-]>0.1 M) the C2 or C3 –OH

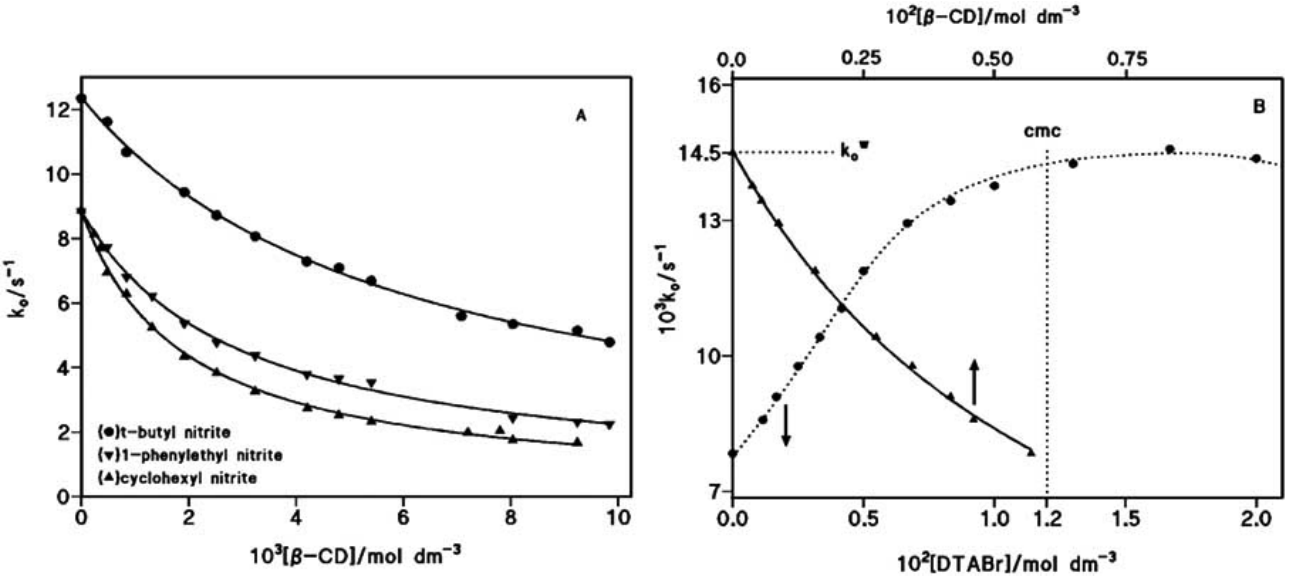


Fig. (8). (A) Variation of k_o as a function of [β-CD] for the hydrolysis of alkyl nitrites in aqueous acid medium (0.014 m HCl) at 25ºC; (B) Variation of k_o as a function of (▲) [β-CD] for the hydrolysis of tert-butyl nitrite in a buffered solution of acetic acid0.030 M of pH 4.89 and of (•)DTABr, dodecyltrimethyl-ammonium bromide, at 5.7 mM of β-CD.

Table 9. Rate Constants Obtained in Water (k_o^w) and in the Presence of 0.010 M β-CD (k_o^c) for the Acid Hydrolysis of RONO at 25 ºC, and Stability Constants of the 1:1 Inclusion Complexes Formed Between RONO·β-CD

R–ONO	k_o^w/s^{-1}	k_o^c/s^{-1}	Inhibition factor	K_c^N/M^{-1}
n-butyl–ONO	7.1[(a)]	4.7	3.6	50
tert-butyl–ONO	12.4[(a)]	4.6	2.7	169
2-ethoxy-ethyl–ONO	12.5×10^{-3}[(b)]	no change	~0	?
n-hexyl–ONO	6.7 [(a)]	2.8	2.4	141
cyclohexyl–ONO	9.0 [(a)]	1.4	6.4	528
cyclohexyl–ONO	9.3×10^{-3}[(b)]	1.2×10^{-3}	7.7	547
2-phenyl-ethyl–ONO	10.2 [(a)]	4.4	4.6	132
1-phenyl-ethyl–ONO	9.2 [(a)]	2.0	4.6	359
1-phenyl-1-propyl–ONO	8.2×10^{-3}[(b)]	1.2×10^{-3}	6.8	593
2-phenyl-2-propyl–ONO	18.5×10^{-3}[(b)]	2.7×10^{-3}	6.8	589
3-phenyl-1-propyl–ONO	7.6×10^{-3}[(b)]	1.7×10^{-3}	4.5	354

[(a)] in aqueous hydrochloric acid 0.014 M; [(b)] in acetic acid/acetate buffer of pH 4.9.

group of a primary CD molecule is ionized, that is, the host is anionic.

Hydrolysis of Alkyl Nitrites

Addition of β-CD to an alkaline solution of RONO results in a strong catalysis giving rise to *saturation kinetics* at high [β-CD], Figs. **9A** and **10A** [103, 104]. At fixed [β-CD] the rate of the basic hydrolysis increases with the $[OH^-]$; nevertheless, the sharp increase (>6-fold) was observed at $[OH^-]<0.1$ M, whereas only a modest (a 1.8-fold on going from 0.1 to 1.0 M of OH^- in the case of ethoxyethyl nitrite) or null (the case of *n*-pentyl nitrite) increase was determined at high OH^- levels. These findings are clear evidence that only the ionized β-CD molecules influence the reaction, in other words, only the inclusion complexes formed between RONO and ionized β-CD react faster than the uncomplexed RONO (the inclusion of RONO with neutral β-CD molecules prevents the hydrolysis). Consequently, at $[OH^-]$ sufficiently high, all β-CD molecules are ionized (pK_a=12.2), then the modest increase in rate upon further increase the $[OH^-]$ is due to the reaction of free RONO catalyzed by OH^- in the bulk water phase. On the other hand, when all RONO molecules are forming inclusion complexes, *i.e.* at high [β-CD], the reaction rate reaches the

maximum value, which depends strongly on the molecular structure of the alkyl nitrite. For example, for the two alkyl nitrites of quite close similar structures, whose results are shown in Figs. **9** and **10**, the maximum catalytic effect of ~15-fold observed for 1-phenyl-1-propyl nitrite enhances to higher than 50-fold when the substrate is 2-phenyl-1-propyl nitrite.

On the other hand, and contrary to that expect, the monomers of DTABr enhance the rate of basic hydrolysis promoted by ionized β-CD, Figs. **9B** and **10B**. As it can be seen, the effect of DTABr is higher for 1-phenyl-1-propyl nitrite than for 2-phenyl-1-propyl nitrite, just the contrary that is found when one compares the effect of only β-CD addition.

As we have already mentioned, the 'normal' expected behavior of DTABr should be an inhibition of the basic hydrolysis (just the opposite for the acid hydrolysis, Fig. **8**) due to that the competition between RONO and DTABr monomers for the β-CD cavity should expel the included RONO, with the consequent retardation of the reaction. There is no doubt that the present results provide an example of *allosteric* activation; *i.e.* substances that are not substrate analogs bind to some site of RONO·β-CD$^-$ (β-CD$^-$ represents ionized β-CD) complex making it much more reactive. The two most plausible possibilities could be the formation of ternary complexes RONO·β-CD$^-$·DTA more reactive than the binary complexes or a particular surrounding of the host-guest complex by DTABr monomers either by displacing the hydration water molecules of the reacting groups (in special for the $-O^-$ group of CD, by increasing thus its nucleophilic character) or by adopting a more proper conformation of the complex.

Scheme **22** accounts for the catalysis by β-CD in the basic hydrolysis of alkyl nitrites, while Table **10** depicts values of parameters along with the calculated values for the quantities k^2 and K_{TS}, which measure the *substrate selectivity* and the *stability of the transition state*, respectively (*vide supra*).

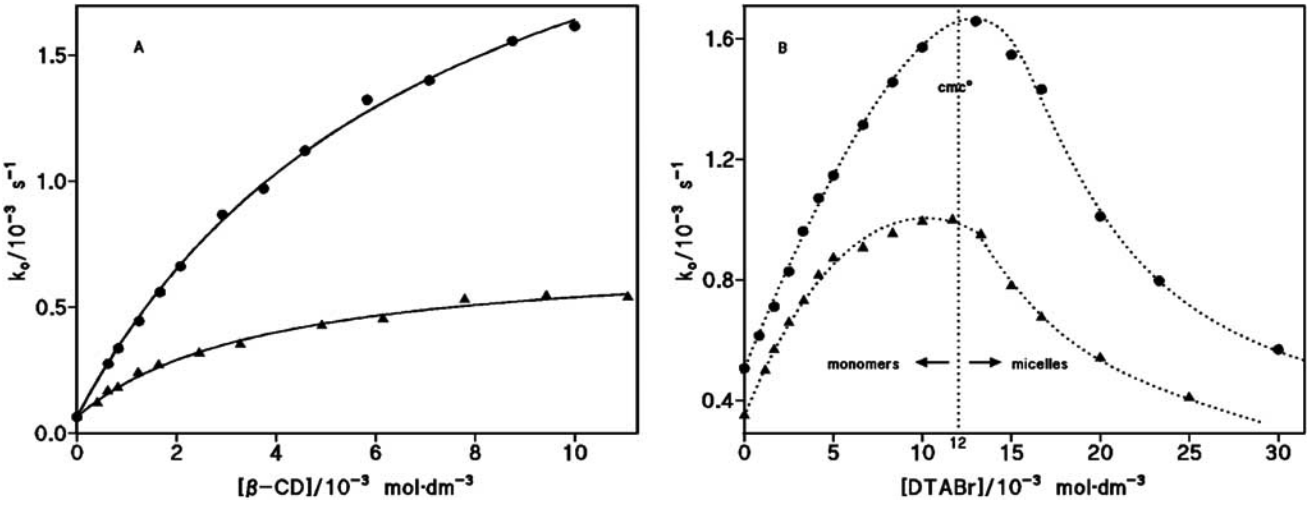


Fig. (9). (A) Catalysis by β-CD in the basic hydrolysis of 1-phenyl-1-propyl nitrite at [OH$^-$]=0.20 M in the absence of DTABr (▲) and in the presence of 0.010 M of DTABr (•); (B) Catalysis by dodecyltrimethyl-ammonium bromide (DTABr) monomers in the basic hydrolysis of 1-phenyl-1-propyl nitrite at [OH$^-$]=0.20 M and fixed [β-CD] equal to (▲)3.7 mM and (•)7.4 mM.

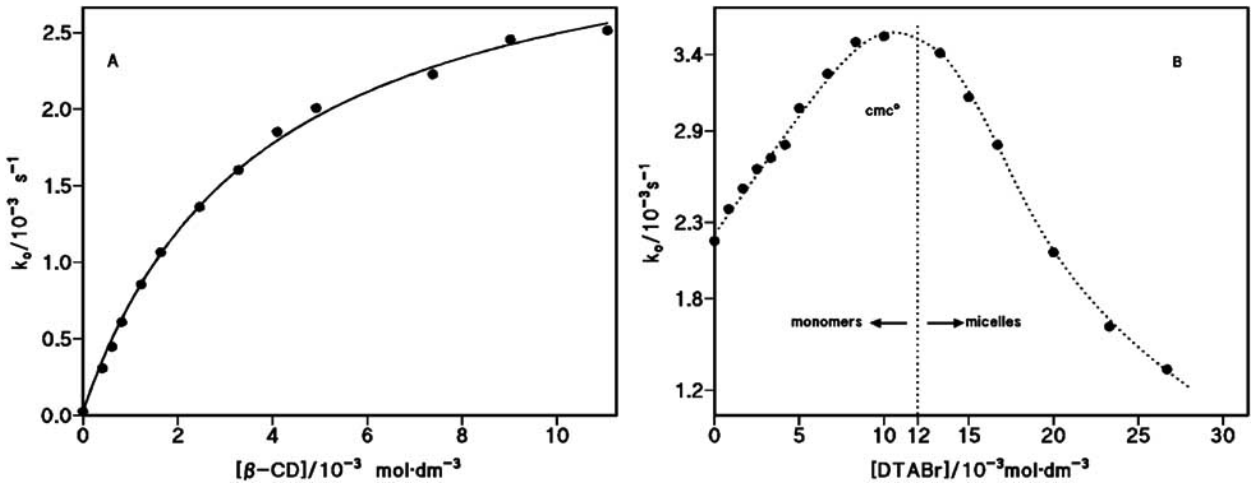
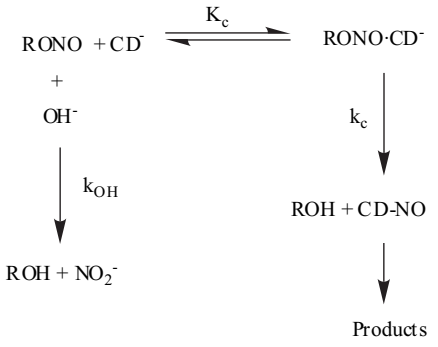


Fig. (10). Basic hydrolysis of 2-phenyl-1-propyl nitrite performed at [OH$^-$]=0.20 M and 25 ºC catalyzed (A) by β-CD and (B) by the monomers of dodecyltrimethylammonium bromide (DTABr) in the presence of a fixed [β-CD]= 7.4 mM.

Table 10. Rate and Equilibrium Constants Obtained in the Alkaline Hydrolysis of Alkyl Nitrites Catalyzed by β-CD at 25 ºC, and Values of the Stability Constants of 1:1 Inclusion Complexes Formed Between Neutral β-CD and Alkyl Nitrites, K_c^N

Alkyl nitrite	k_o^w/ s^{-1} (a)	k_c/s^{-1}	$k^2/M^{-1}s^{-1}$ (b)	K_c/M^{-1}	K_{TS}/M^{-1} (c)	K_c^N/M^{-1}
n-butyl nitrite	$\sim 2\times10^{-5}$	2.0×10^{-3}	0.10	51	5200	50
n-pentyl nitrite	$\sim 1.5\times10^{-5}$	2.1×10^{-3}	0.105	50	7000	141
cyclohexyl nitrite	1.6×10^{-5}	0.3×10^{-3}	0.084	280	5250	547
2-ethoxyethyl nitrite	1.8×10^{-4}	4.0×10^{-3}	0.26	65	1642	unknown
1-phenylethyl nitrite	1.2×10^{-4}	1.6×10^{-3}	0.18	114	1500	282
2-phenylethyl nitrite	3.5×10^{-5}	5.5×10^{-3}	0.55	100	15700	132
1-phenyl-1-propyl nitrite	7.6×10^{-5}	0.6×10^{-3}	0.16	270	2395	580
3-phenyl-1-propyl nitrite	5.6×10^{-5}	2.5×10^{-3}	0.75	301	21760	354
2-phenyl-1-propyl nitrite	6.5×10^{-5}	3.4×10^{-3}	0.90	265	13850	430
2-phenyl-2-propyl nitrite	$<1\times10^{-5}$	No effect	---	unknown		589

(a) $k_o^w = k_{OH}[OH^-]$; (b) $k^2 = k_c K_c$; (c) $K_{TS} = k^2/k_o^w$.



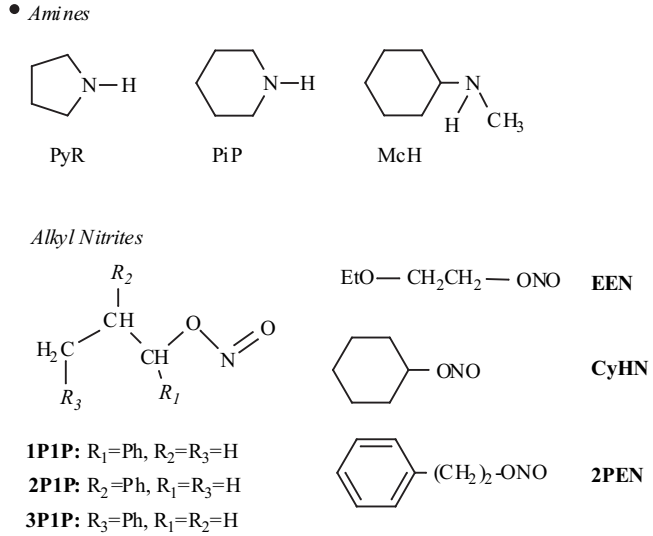
Scheme 22. Proposed reaction scheme for the catalysis of β-CD in the alkaline hydrolysis of alkyl nitrites.

One might note the high sensitivity of K_{TS} to the structural changes in the alkyl nitrite, a typical characteristic of *covalent catalysis* by cyclodextrins; in the same sense, lied the k^2 values, high substrate selectivity corresponds also with high stability of the transition state.

For comparison purposes, entry 7 of table 10 lists K_c^N values, *i.e.* the stability constants of 1:1 inclusion complexes formed between *neutral* β-CD and RONO, which are higher than the corresponding values with *ionized* β-CD (K_c). Taking into account that the nature of RONO is the same as in acid as in alkaline medium, the lowering of K_c in comparison to K_c^N reflects the difficulty in analysing the driving forces of inclusion. The most remarkable differences between both situations are the higher hydration degree of ionized $-O^-$ groups of CD, in which case the highly ordered water molecules that are surrounding them need to be expelled out −at least in part− in the process of RONO inclusion, and the possibility of RONO forms hydrogen-bonds with the OH-groups of the wider rim of neutral CD. Both effects operate in the same direction, and this could be the reason of the large difference between K_c and K_c^N.

Nitrosation of Amines

The nitrosation of the secondary amines by the alkyl nitrites, displayed in Scheme **23**, has been studied in basic medium of β-CD solutions. The acidity of the aqueous solutions was controlled by using solution buffers of the own amine (pH~11) or a strong alkaline medium ($[OH^-] \geq 0.1$ M) [103,104,106].



Scheme 23. Structures of the amines and alkyl nitrites studied in the transnitrosation reaction mediated by β-CD in basic media.

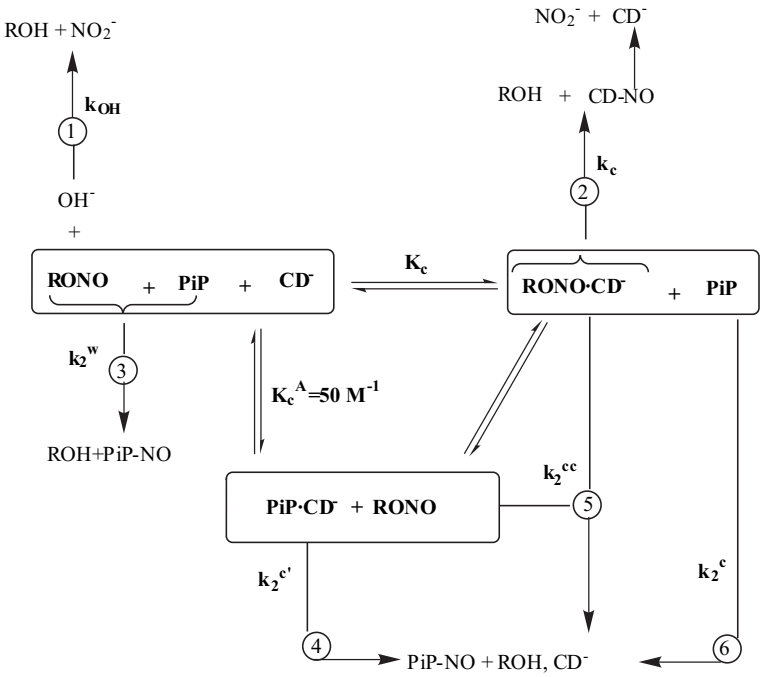
All these alkyl nitrites have proved to form inclusion complexes with either neutral or ionized β-CD. In the same sense, the unprotonated form of these amines forms inclusion complexes with β-CD; the corresponding stability constants have been measured as 6, 50, and 550 M^{-1} for pyrrolidine, piperidine, and N-methylcyclohexylamine, respectively [107,108]. The observed effect caused by the

Table 11. Nature of the Observed Effect of β-CD in the Nitrosation Reaction of Amines by Alkyl Nitrites in Basic Media. The Figures in Parentheses Represent the Quantitative Effect (Factor), Determined as the Ratio of k_o Measured in the Absence and Presence of Approximately 0.01 M of β-CD; the Range (Last Entry) is Obtained for Different Amine Concentrations: the Higher Value is Observed at the Lower [Amine] (~1.7 mM)

amine	RONO	effect (factor)	amine	RONO	effect (factor)
Buffered solutions of the amine (pH ~11)			*Alkaline medium, [OH^-]=0.10 or 0.20 M*		
PyR	EEN	No effect	PyR	EEN	Catalysis (×1.4)
PyR	1P1P	Inhibition (:6)	PyR	CyHN	Inhibition (:1.7)
PyR	3P1P	~no effect	PyR	1P1P	Inhibition (:1.7-2)
---	--	---	PyR	2PEN	Catalysis (×1.5)
---	---	---	PyR	2P1P	Catalysis ×(2-4)
---	---	---	PyR	3P1P	Catalysis ×(2 –6)
PiP	1P1P	Inhibition (:5)	PiP	EEN	Catalysis (×5)
PiP	2P1P	Catalysis (×1.2)	PiP	2P1P	Catalysis ×(5-11)
---	---	---	PiP	3P1P	Catalysis ×(11-16)
---	---	---	PiP	1P1P	Inhibition (:1.2)
---	---	---	MCH	EEN	Catalysis (×3)
---	---	---	MCH	1P1P	Catalysis (×2.5)
---	---	---	MCH	2P1P	Catalysis ×(4.5-7)
---	---	---	MCH	3P1P	Catalysis ×(15-30)

presence of β-CD on the *transnitrosation* reaction between these amines and alkyl nitrites was either an inhibition, no effect, or a catalysis. As the nature the degree of the observed effect depends not only on the type of both amine and alkyl nitrite, but also on the conditions of the reaction medium. The gamut of different behaviors are depicted in Table **11**.

The magnitude of the effect estimated as the ratio of k_o^{max}/k_o^w (with k_o^{max} and k_o^w being the observed rate constants measured at ca. [β-CD]=0.01M and in the absence



Scheme 24. Reaction pathways that have to be considered in the analysis of the effect of β-CD in the nitrosation of amines in alkaline medium.

of β-CD, respectively) firstly, decreases as the amine concentration increases, and secondly, depends strongly on the amine; the highest catalysis is observed with McH whereas the lowest, with PyR. The first observation is consequence of the competitive reaction *via* both uncomplexed amine and alkyl nitrite whose rate increases in proportion to the amine concentration. The second observation suggest that the reaction *via* both complexed reagents (RONO·CD+amine·CD) must be significant, which is supported by the finding that the reciprocal plots of k_o against [β-CD] describe up-curves.

A quantitative description of all kinetic findings could be done on the basis of the reaction Scheme **24** put forward the case of PiP, where, besides the basic hydrolysis reaction *via* either OH^- (step 1) or complexed RONO to ionized β-CD (step 2), the possibility of nitrosation reactions between free RONO and free amine (setp 3), or between complexed RONO and free amine (step 4) or *vice versa* (step 5) –both steps are kinetically indistinguishable–, and finally between both complexed reagents (step 5) are considered. From this general reaction scheme the following simplifications can be made.

1. Buffer Solutions of the Amine (pH~11)

Under these experimental conditions, steps 1 and 2 do not take place, because both OH^- and ionized β-CD concentrations are too small. In the same sense, in using low PyR concentration (<0.05 M), the quantity of complexed amine is negligible due to the low value of K_c^A =6 M^{-1}; therefore, steps 4 and 5 are not detected. Even in the case of PiP, which has higher K_c^A (= 50 M^{-1}), the step 5 is not kinetically observed as a consequence of the structure postulated for the corresponding transition state, which requires a water molecule to fix both reagents, vide infra, but this alternative is only possible with ionized β-CD.

2. Alkaline Medium ([OH^-] >0.1 M)

Taking into account that the nitrosation is much faster than the alkaline hydrolysis, in alkaline medium the step 1 is really important only with activate alkyl nitrites, e.g. EEN, and step 2 becomes only significant at high β-CD, when an important amount of the alkyl nitrite is forming inclusion complexes. For the same reasons given above, in the case of the nitrosation of PyR, both steps 4 and 5 do not need to be considered, that is, the reaction scheme for the nitrosation of pyrrolidine in the presence of β-CD considers only both hydrolysis steps, 1 and 2, as well as the nitrosation reaction through both uncomplexed reagents, step 3, or complexed RONO with free PyR, step 6.

Table **12** lists values of the rate constants that appear in Scheme **24**. For the same amine, the most reactive alkyl nitrites appear to be EEN and 1P1P as a consequence of the high stability of the leaving alkoxide, which is stabilized by

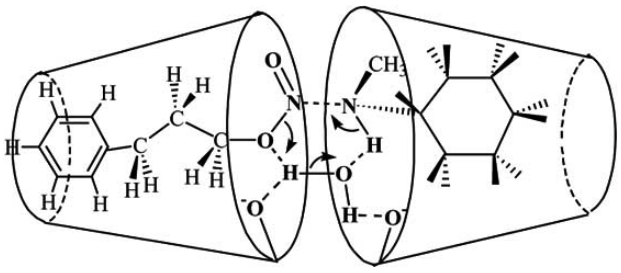
Table 12. Reaction Rates Between the Amines PyR, PiP and MCH and Alkyl Nitrites (RONO) Obtained in Aqueous Buffered Solutions of the Amine and in Alkaline Medium in the Absence and Presence of β-CD. (See Scheme 24 for the Meaning of the Parameters)

	Amine-buffer medium (neutral β-CD)			*Alkaline medium (ionized β-CD)*		
RONO	k_2^w/ $M^{-1}s^{-1}$	K_c^N /M^{-1}	k_2^c /$M^{-1}s^{-1}$	K_c /M^{-1}	k_2^c /$M^{-1}s^{-1}$	k_2^{cc} /$M^{-1}s^{-1}$
Pyrrolidine (PyR), K_c^A= 6 M^{-1}						
EEN	1.36 /1.46	---	---	65	1.6	---
CyHN	0.82	547	?	275	0.096	---
2PEN	0.84	132	?	92	1.09	---
1P1P	1.07	528	0.050	269	0.34	---
2P1P	0.69	?	?	280	0.66	---
3P1P	0.30	355	0.24	310	0.34	---
Piperidine (PiP), K_c^A= 50 M^{-1}						
EEN	0.33	---	---	65	0.40 [(a)]	2.0
1P1P	0.20	590	0.030 [(a)]	260	0.04 [(a)]	0.20
2P1P	0.12	430	0.21 [(a)]	280	0.28 [(a)]	0.92
3P1P	0.050	354	?	310	0.15 [(a)]	0.88
N-methyl-cyclohexylamine (McH), K_c^A= 550 M^{-1}						
EEN	0.170	---	---	65	0.46 [(a)]	0.50
1P1P	0.092	550	?	264	0.16 [(a)]	0.093
2P1P	0.055	580	?	273	0.53 [(a)]	0.30
3P1P	0.028	354	?	310	0.36 [(a)]	0.26

[(a)]Values of k_2^c or $k_2^{c'}$ because they are kinetically indistinguishable.

electron withdrawing substituents. By contrast, the complexed 1P1P shows to be the lowest reactive; the possible reason resides in a deeper protruding of this alkyl nitrite into the β-CD cavity, which forces the NO-transfer to take place in a low polar microenvironment. This fact accounts for (i) the strong inhibition found in the nitrosation of PyR –an amine with low tendency to form inclusion complexes– either in a buffer solution of pyrrolidine-pyrrolidinium chloride or in alkaline medium, and (ii) the much lower values of both k_2^c and k_2^{cc} found in the nitrosation of the other amines, piperidine of N-methylcyclohexyl amine in comparison with e.g. the values determined for the same rate constants in the nitrosation by EEN or 3P1P.

The structures of these two latter alkyl nitrites can be considered as extended molecules, that protrude lengthwise into the β-CD cavity. This configuration leaves the –NO group quite close the surface of the cavity, a hydrated region, and because of that the reactivity is much higher. In the same sense, it is possible to explain the higher values of k_2^{cc} that k_2^w, which means that an important decrease in the *activation energy* of the reaction *via* step 5 should occur. A possible explanation could be the formation of *channel-like structures* that, besides serving to fix both reagents in close proximity, might intervene in the formation of the *transition state*. The ionized $-O^-$ group of a β-CD molecule will form H-bonds with either water molecules or with the amine included into the β-CD cavity. This special arrangement of the reactants in the *activated complex*, shown in Scheme **25**, with a water molecule participating in the fixation of the reagents to the ionized host, should result in a strong decrease in the activation energy, even though the gain in entropy will not be negligible. The proposed structure for the transition state also explains the absence of the reaction through both complexed reactants when the reaction is studied in a buffer of the amine, that is to say in conditions of non-ionized β-CD, in which case the possibility of H-bonding formation between the -OH groups of β-CD and the amine, or a water molecule, is much more constrained.



Scheme 25. Possible arrangement of the molecules of 3P1P nitrite and McH amine in the transition-state corresponding to the reaction *via* both complexed reagents with β-CD.

5. CONCLUDING REMARKS

Cyclodextrins are now widely used in many different fields, and they are being widely applied in developing the chemistry at molecular level of the future.

The chemistry of nitroso compounds becomes in the last decade a topic of growing interest. The reactions undergo by the different kinds of nitroso compounds including N-nitroso, C-nitroso, O-nitroso, S-nitroso, or nitrosyl metal complexes are examined in detail from both the chemical and biological point of views. The kinetics and mechanisms of NO-transfer are the matter of many studies and discussions, since a new and unexpected role for NO –the molecule of the Year 1992 (Koshland, D. E. *Science*, **1992**, *258*, 1861)– as a key physiological regulator has been revealed.

In this review I have tried to connect both concepts: cyclodextrins and nitroso compounds, by focusing attention on kinetic and mechanistic effects of cyclodextrins in nitrosation chemistry. In particular, regarding kinetics, I spoke about the effects of cyclodextrins in systematic variations in the experimental conditions of the reaction media, structure and nature of the nitroso compound or of the nitrosatable substrate, as well as on the nature of the studied reaction, in fact, acid and basic hydrolysis, nitrosation and transnitrosation (electrophile↔nucleophile interaction) reactions have all been analysed. The promising results undoubtedly are important for physicochemical studies relative to the molecular behavior in solution that concern crucial aspects in catalysis to continue.

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