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Fast Atom Bombardment Mass Spectrometric and Tandem Mass Spectrometric Investigation in Thioglycerol on Protonated Non-covalent Associations of β-Cyclodextrin with 2-Acetyl, 2-Propionyl-1-pyrroline and 5-Acetyl-2,3-dihydro-1,4-thiazine, Roast Smelling Odorants in Food. Role of the Matrix

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2-Acetyl-1-pyrroline (1), 2-propionyl-1-pyrroline (2) and 5-acetyl-2,3-dihydro-1,4-thiazine (3), roast smelling odorants in food, form stable inclusion compounds with β-cyclodextrin. Fast atom bombardment (FAB) mass spectra of such complexes in thioglycerol showed abundant $[G + Hs + Mx + H]^+$ ions, where G = guest (1, 2 or 3), Hs = host (β-cyclodextrin) and Mx = one molecule of matrix, consistent with protonated non-covalent three-component adducts, and nearly negligible 1:1 associations of the type $[G + Hs + H]^+$. Collision-activated decomposition (CAD) experiments indicated that $[G + Hs + Mx + H]^+$ are made of neutral β-cyclodextrin and protonated 1:1 guest–matrix adducts. The nature of these latter adducts was investigated by FAB mass spectrometric experiments on 1, 2 and 3 in thioglycerol without β-cyclodextrin. In all cases the most intense signals are due to $[G + Mx + H]^+$ and $[G + H]^+$, with a small contribution of $[G + Mx - H_2O + H]^+$ to the total ion current. CAD experiments on $[G + Mx + H]^+$ afforded protonated guest molecules as the base peak, consistent with the decomposition of protonated non-covalent 1:1 guest–matrix associations, possibly mediated by an intermolecular hydrogen bond. According to these data, there is a significant contribution of non-covalent three-component associations to $[G + Hs + Mx + H]^+$ complexes, although the possibility of the formation of covalent guest–matrix adducts is not ruled out definitely, as discussed in the text. © 1997 by John Wiley & Sons, Ltd.

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INTRODUCTION

Molecular encapsulation of compounds with biological activity by water-soluble and naturally available hosts such as β-cyclodextrin (β-CD) covers a variety of different interests, from structural investigations of non-covalent interactions to industrial exploitation. The phenomenon of inclusion of guest molecules within the hydrophobic cavity of β-CD has been extensively reviewed.[1] Traditionally, the structural characterization of the inclusion compounds in terms of stoichiometry, geometry of binding, nature of the interactions and exchange equilibria rely upon diffraction[2] and NMR[3] methods. More recently, mass spectrometry (MS) with the use of soft ionization techniques such as ionspray (IS), electrospray (ESI) and fast atom bombardment (FAB), allowed the study of non-covalent complexes of several kinds of host molecules as gaseous ions,[4] including complexes of natural and chemically modified cyclodextrins.[5–13]

Established applications of β-CD inclusion complexes concern the pharmaceutical industry, and are aimed at improving the bioavailability of barely water-soluble drugs and reducing unwanted side-effects. Innovative applications are found in food chemistry and industry: molecular encapsulation of flavours in malto- and/or cyclodextrins is expected to give them long-lasting properties, therefore improving the quality of the finished goods. In a previous paper,[14] the enzyme-mediated synthesis of 2-acetyl-1-pyrroline (**1**) and 2-propionyl-1-pyrroline (**2**), roast smelling odorants present in food, was reported along with the preparation of water-soluble inclusion compounds of **1** and **2** with β-CD and preliminary characterizations via NMR and FABMS.

In this paper, we report a detailed FABMS and

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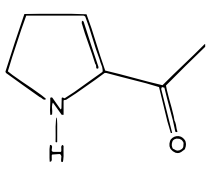

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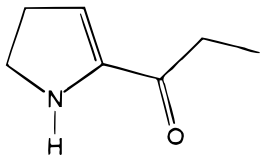

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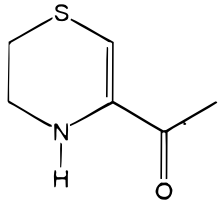

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MS/MS study of the inclusion complexes of **1**, **2** and another related roast smelling odorant, 5-acetyl-2,3-dihydro-1,4-thiazine (**3**),[15] with β-CD, using thioglycerol as a liquid matrix. The possible role of this matrix as a chelating agent able to generate stable non-covalent adducts with protonated **1**, **2** and **3**, with the consequent formation of non-covalent multicomponent association with β-CD, is discussed.

EXPERIMENTAL

Sample preparation

Compounds **1**, **2** and **3** were synthesized as reported previously.[14,15] β-CD was purchased from Roquette and used as received (water content $\sim$5% w/w). Commercial 3-mercaptopropane-1,2-diol (thioglycerol) was used as a matrix. Host–guest complexes of **1** and **2** were prepared by mixing a CH_2Cl_2 solution of the guest molecule with β-CD previously dissolved in the matrix and evaporating the volatile solvent by gently heating the FAB tip. The complex of **3** with β-CD was prepared by kneading the solid host and guest together and dissolving the mixture in the appropriate amount of matrix. In all cases the overall complex concentration in the matrix ranged between 0.05 and 0.01 M. Other matrixes such as glycerol and 3-nitrobenzyl alcohol gave poor spectra.

Mass spectrometry

FABMS and MS/MS measurements were made on a Finnigan-MAT TSQ70 triple-stage quadrupole instrument equipped with an Ion-Tech (Teddington, UK) atom gun with Xe as bombarding gas. The atom gun operating conditions were emission current 2 mA and accelerating voltage 8 keV. In all the experiments the source was kept at room temperature. CsI was used for mass calibration. Collision-activated decomposition (CAD) was achieved by using the first quadrupole as the parent mass selector, the second quadrupole as the collision cell (CA gas argon, $p = 8 \times 10^{-4}$ Torr (1 Torr = 133.3 Pa), nominal collision energy $E_{lab} = 10$ eV) and the third quadrupole as the mass analyser (typically, m/z scan range 100–1600, scan rate 500 u s^{-1}). Unimolecular decomposition experiments were carried out by setting the instrument in the MS/MS mode without CA gas in the second quadrupole. All the spectra were acquired in the centroid mode and calculated over an average of 20–60 scans using standard Finnigan software.

RESULTS AND DISCUSSION

The FAB mass spectra of the complexes of **1**, **2** and **3** with β-CD in thioglycerol are displayed in Fig. 1 and show some common features that can be summarized as follows: (i) there are fairly intense peaks at m/z 1354, 1368 and 1386 for **1**, **2** and **3**, respectively, consistent with the formation of protonated three-component adducts of general structure $[G + Hs + Mx + H]^+$,

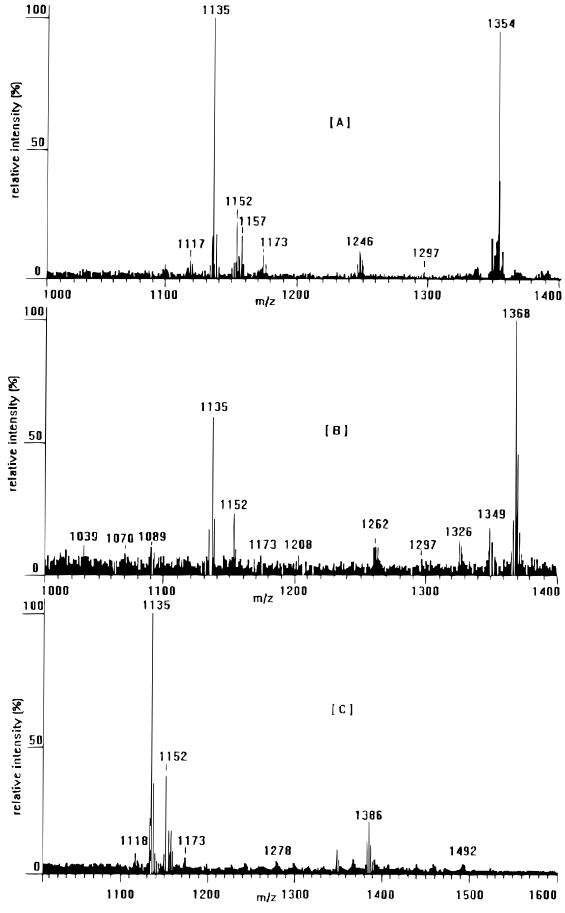

Figure 1. FAB mass spectra of β-CD inclusion complexes with (A) **1**, (B) **2** and (C) **3**.

where G = guest (**1**, **2** or **3**), Hs = host (β-cyclodextrin) and Mx = one molecule of matrix; (ii) the signals attributed to protonated 1:1 host–guest complexes $[G + Hs + H]^+$ (*m/z* 1246, 1262 and 1278 for **1**, **2** and **3**, respectively) are noticeably less intense than those of the corresponding protonated ternary associations $[G + Hs + Mx + H]^+$; (iii) in the experimental scan range (1000–1600 u) a strong signal due to the protonated host, *m/z* 1135, is detected in all cases (relative abundance 100, 62 and 100% for **1**, **2** and **3**, respectively), with less intense signals due to ammonium-cationized $[Hs + NH_4]^+$ (*m/z* 1152), sodium-cationized $[Hs + Na]^+$ (*m/z* 1157) and, for **1** and **3** only, potassium-cationized host $[Hs + K]^+$ (*m/z* 1173). The *m/z* 1349 and 1366 peaks are due to β-CD–matrix clusters, already observed in the FAB mass spectra of the β-cyclodextrin–piroxicam inclusion complex.[10]

We tried to gain more information on the structure of $[G + Hs + Mx + H]^+$ associations by performing a series of MS/MS experiments from which we could reasonably expect: (i) to shed some light on the nature and relative strength of the interactions binding the three components, with specific emphasis on the host–guest partners within such adducts, according to what has been reported recently (see below) for other gaseous charged host–guest multicomponent systems,[12,13] and (ii) to elucidate, in the present case, the role played by the FAB matrix in the formation of the complexes. The role of the matrix should not be underestimated, especially when dealing with FAB liquid secondary ion mass spectra of putative non-covalent associations. Indeed, it is well assessed that beam-induced energy release can generate highly reactive species, such as electrons, radical ions and excited-state species, which may undergo chemical reactions with the analyte and/or the matrix.[16–18] The ions thus originated appear as artefacts in the spectrum and may lead to deceptive conclusions on the structure of the analyte.

In a first set of experiments, we observed the CAD of the protonated three-component species $[G + Hs + Mx + H]^+$. The results are summarized in Table 1. The most abundant fragment ions correspond to the protonated 1:1 association of guest and thioglycerol (*m/z* 220, 234 and 252 for **1**, **2** and **3**, respectively), with less abundant ions resulting from the latter with loss of one water molecule. The *m/z* 1135 peak of protonated β-CD was not detectable in any case. In a similar fashion, unimolecular decomposition (see Experimental for details) of $[G + Hs + Mx + H]^+$ yielded the same fragment ions $[Mx + G + H]^+$ as obtained in CAD experiments, with the only obvious difference of the relative abundance with respect to the parent. These experimental results are consistent with a relatively tight 1:1 association of the guest molecules with thioglycerol and a weak interaction of the latter 1:1 association with neutral β-cyclodextrin. The problem of the nature of what we have generically referred to as '1:1 association' of guest and thioglycerol should be correctly addressed at this stage. There may be at least two reasonable hypotheses: (i) the association is actually a covalent compound of thioglycerol and **1**, **2** and **3** generated during the bombardment (1,4-addition for **4** or 1,2-addition for **5**, Fig. 2), according to the reactivity of the α,β-unsaturated C=O system towards sulphur nucleophiles and radicals;[19,20] (ii) protonated pyrrolines or thiazine and neutral thioglycerol are bound together by intermolecular hydrogen bonds, possibly with a bridgehead H^+, giving rise to a non-covalent adduct (**6**, Fig. 2). A similar scheme was recently proposed by Madhusudanan[21] to rationalize the formation of $[M + matrix + H]^+$ ions in the FABMS of dihydroxybenzenes using 3-nitrobenzyl alcohol as a liquid matrix. With the aim of obtaining more information on the nature of the association of guest and matrix, we investigated the behaviour of **1**, **2** and **3** in thioglycerol without β-cyclodextrin. The results of FABMS and MS/MS experiments on isolated **1**, **2** and **3** are summarized in Tables 2 and 3. The 2-acyl-1-pyrrolines examined gave abundant protonated 1:1 adducts with thioglycerol, together with a fairly large amount of protonated acylpyrroline. Compound **3** afforded similar

Table 1. FABMS/MS data for β-CD inclusion complexes of compounds 1, 2 and 3[a]

	$[Mx + G + H]^+$ *m/z* (rel. int., %)	$[Mx + G - H_2O + H]^+$ *m/z* (rel. int., %)	$[G + H]^+$ *m/z* (rel. int., %)	Others *m/z* (rel. int., %)
[**1** + Hs + G + Mx + H]$^+$ 1354 (9)[b]	220 (100)	202 (13)	—	—
[**1** + Hs + G + Mx + H]$^+$ 1354 (100)[c]	220 (21)	—	—	—
[**2** + Hs + G + Mx + H]$^+$ 1368 (8)[b]	234 (100)	216 (4)	—	—
[**2** + Hs + G + Mx + H]$^+$ 1368 (100)[c]	234 (13)	—	—	—
[**3** + Hs + G + Mx + H]$^+$ 1386 (31)[b]	252 (100)	234 (6)	—	325 (12)[d]
[**3** + Hs + G + Mx + H]$^+$ 1386 (100)[c]	252 (11)	—	—	—

[a] The first column gives the chemical structure of the parent ion, the nominal mass and the relative intensity (%). The other columns gives the chemical structure, *m/z* and relative intensity (%) of the observed fragment ions. Abbreviations: Hs = host; G = guest; Mx = matrix.
[b] CAD gas: Ar, p = 0.8 mTorr.
[c] No CAD gas.
[d] Fragmentation product of β-cyclodextrin, corresponding to protonated $C_{12}H_{20}O_{10}$.

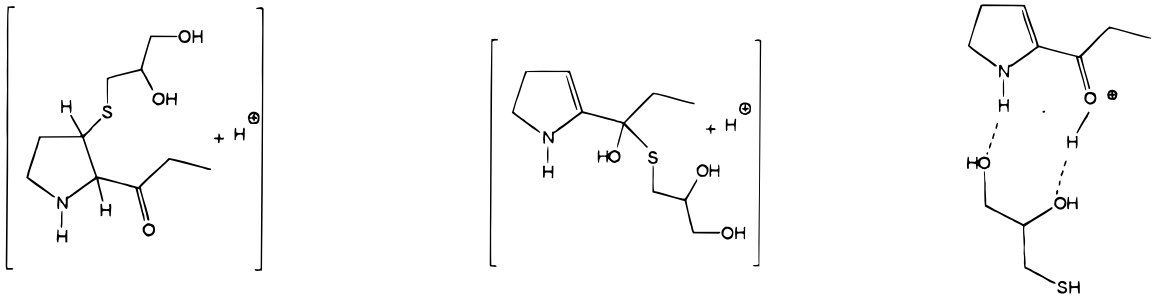

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Figure 2. Possible structures of protonated 1 : 1 adducts of **2** with thioglycerol: 1,4-adduct (**4**); 1,2-adduct (**5**); non-covalent adduct (**6**).

peaks, but with two remarkable differences: (i) a greater abundance of $[G + H]^+$ (*m/z* 144) with respect to $[G + Mx + H]^+$ (*m/z* 252) (see the last column in Table 2) and (ii) a large contribution of the *m/z* 143 radical cation $G^{+\cdot}$ to the total ion current, not present for **1** and **2**. Unimolecular decomposition of the parent $[G + Mx + H]^+$ gave in all the cases $[G + H]^+$ ions and CAD experiments on the same parents yielded

Table 2. FABMS data for guest molecules 1, 2 and 3[a]

Guest	$[G + Mx + H]^+$ *m/z* (rel. int., %)	$[G + Mx - H_2O + H]^+$ *m/z* (rel. int., %)	$[G + H]^+$ *m/z* (rel. int., %)	$G^{+\cdot}$ *m/z* (rel. int., %)	Ratio $[G + Mx + H]^+/[G + H]^+$
1	220 (100)	202 (20)	112 (62)	—	1.61
2	234 (100)	—	126 (100)	—	1.00
3	252 (41)	—	144 (100)	143 (63)	0.41

[a] Scan range: *m/z* 1000–1600. All the reported masses are nominal. Abbreviations: Hs = host; G = guest; Mx = matrix.

Table 3. FABMS/MS data for guest molecules 1, 2 and 3[a]

	$[Mx + G - H_2O + H]^+$ *m/z* (rel. int., %)	$[G + H]^+$ *m/z* (rel. int., %)	Others *m/z* (rel. int., %)
$[\mathbf{1} + Mx + H]^+$ 220 (100)[c]	202 (2)	112 (5)	—
$[\mathbf{2} + Mx + H]^+$ 234 (61)[b]	216 (10)	126 (100)	119 (2);[d] 98 (3);[e] 70 (2)[f]
$[\mathbf{2} + Mx + H]^+$ 234 (100)[c]	216 (0.3)	126 (4)	—
$[\mathbf{3} + Mx + H]^+$ 252 (56)[b]	234 (7)	144 (100)	102 (31);[g] 74 (3)[h]
$[\mathbf{3} + Mx + H]^+$ 252 (100)[c]	—	144 (4)	—

[a] The first column gives the chemical structure of the parent ion, the nominal mass and the relative intensity (%). The other columns give the chemical structure, *m/z* and relative intensity (%) of the observed fragment ions. Abbreviations: G = guest; Mx = matrix.
[b] CAD experiment (Ar, $p = 0.8$ mTorr).
[c] Unimolecular decomposition, no CAD gas.
[d] Corresponding to $[C_4H_7O_2S]^+$, protonated formate ester of anhydrothioglycerol, as confirmed by MS/MS on *m/z* 234.
[e] Corresponding to $[C_6H_{12}N]^+$, protonated 2-ethyl-1-pyrroline by loss of CO from protonated **2**, as confirmed by MS/MS on *m/z* 126.
[f] Corresponding to $[C_4H_8N]^+$, protonated pyrroline by loss of methylketene from protonated **2**, as confirmed by MS/MS on *m/z* 126.
[g] Corresponding to $[C_4H_7NS]^+$, protonated 2,3-dihydro-1,4-thiazine obtained by loss of neutral ketene from protonated **3**, as confirmed by MS/MS on *m/z* 144.
[h] Corresponding to $[C_2H_4NS]^+$, possibly by loss of neutral methyl vinyl ketone from protonated **3**, consistent with MS/MS on *m/z* 144.

$[G + H]^+$ as the base peak for all the guest molecules. Both FABMS and MS/MS experiments indicated that the contribution of $[G + Mx - H_2O + H]^+$ to the total ion current is small compared with $[G + Mx + H]^+$. Abundant water loss is expected in the fragmentation pattern of protonated species **5**, due to the presence of a tertiary OH group. The most straightforward way of fragmenting **6** is breaking the intermolecular hydrogen bond with release of neutral thioglycerol and detection of $[G + H]^+$, whilst either neutral water loss or neutral thioglycerol release (as a product of retro-addition to the C=C double bond) seem to be reasonable as far as the fragmentation of **5** is concerned. The present data are consistent with the participation of both covalent and non-covalent mechanisms in the formation of guest–matrix association. Indeed, it is undeniable that non-covalent associations play a role, either with or without β-cyclodextrin. Notably, the fragmentation pattern of *m/z* 252 also shows *m/z* 102 (31% relative abundance), assigned to protonated 2,3-dihydro-1,4-thiazine and confirmed by CAD of *m/z* 144, obtained from protonated **3** via loss of neutral ketene. In a similar fashion, *m/z* 70 is obtained in the CAD of [**2** + thioglycerol + H]$^+$ via loss of methylketene.

This ensemble of results on the isolated guests molecules supports the hypothesis of gaseous associations between protonated **1**, **2** or **3** and neutral thioglycerol acting as a chelating agent and tends to rearrange a possible covalent association of the matrix with the analyte molecules. A relevant consequence is that an appreciable contribution to $[G + Hs + Mx + H]^+$ is provided by genuine three-component non-covalent associations.

It is worth making a comparison between these data and the other examples of ternary non-covalent associations of β-CD inclusion complexes reported recently.[12,13] Particularly stable ternary complexes such as β-CD–terfenadine (TFN) and organic hydroxy acids (tartaric or citric acid, HA) were studied[12] in the gas phase by ionspray MS and MS/MS, and more recently ternary adducts of β-CD with various drugs and diethanolamine (DEA) were characterized under similar MS and MS/MS conditions.[13] The CAD of the protonated 1:1:1 associations gave rise to protonated 1:1 TFN–β-CD and to protonated 1:1 DEA–β-CD products, respectively. These fragmentation patterns, characterized by the loss of neutral HA or guest molecule, respectively, were interpreted as a consequence of the relative difference in the intensities of the binding interactions among the components of the gaseous charged multicomponent associations.[13] The MS/MS results of the present work on the ternary adducts of **1**, **2** and **3** showed the loss of the host β-CD as a neutral moiety, thus providing a complementary pattern to those mentioned above, and indicating that, in the present cases, the non-covalent 1:1 interactions between the guest molecules and one molecule of matrix are stronger than those responsible for the binding of these two components with β-CD.

Moreover, the low abundance of the protonated 1:1 guest–β-CD complex in the FAB mass spectra (see Fig. 1) suggests that the presence of the matrix as a third, highly polar component contributes effectively to the formation of fairly stable, charged, non-covalent ternary adducts. Thus, thioglycerol is playing the double role of a matrix for FAB ionization and a chelating reagent for complexation. MS/MS experiments carried out on the parent $[Hs + G + H]^+$ provided further evidence. Indeed, fragmentation of the parent ions formally corresponding to protonated 1:1 host–guest adducts of **1** (*m/z* 1246), **2** (*m/z* 1262) or **3** (*m/z* 1278) afforded completely different results to those of the related 1:1:1 adducts $[G + Hs + Mx + H]^+$. Collision of *m/z* 1246 and 1262 did not show any intelligible signal above the background noise, whilst the CAD of *m/z* 1278 resulted in a collection of fragments of general formula $[\beta\text{-CD} - nH_2O + H]^+$, with $n = 2$, 3, 4 (42% relative abundance each) or 5 (100% relative abundance), plus *m/z* 163 (14%), corresponding to $[\text{glucose} - H_2O + H]^+$, and only 11% of *m/z* 1135, corresponding to protonated β-CD. These data indicate that *m/z* 1278 is mainly generated by a covalent adduct of β-CD and the guest molecule, with only a negligible contribution of a 1:1 non-covalent host–guest association. The highly reactive odd electron ion $\mathbf{3}^{+\cdot}$ generated by the bombardment process (see above) is likely to form covalent bonds with neutral cyclodextrin, either in the liquid matrix or in the selvedge region, thus giving rise, under CAD conditions, to fragmentation of the oligosaccharide skeleton. Indeed, as we have already demonstrated with FABMS experiments on 1:1 inclusion complexes of β-CD and piroxicam,[10] the fragmentation of cyclodextrin is not likely to occur under MS/MS conditions in the presence of a weak, non-covalent complex.

CONCLUSIONS

FABMS and MS/MS results on $[G + Hs + Mx + H]^+$ and isolated guests suggest the existence of a hierarchy of non-covalent interactions controlling the formation and decomposition of the various adducts: (i) a relatively tight association of matrix and guests affording $[G + Mx + H]^+$, possibly mediated by an intermolecular hydrogen bond with bridgehead H^+ (although beam-induced chemical reactions between matrix and guest leading to covalent adducts should not be ruled out), and (ii) a much weaker attractive interaction between neutral β-CD and $[G + Mx + H]^+$, involving van der Waals forces between the lipophilic cavity of cyclodextrin and the apolar parts the guest molecule. The formation of a non-covalent three-component adduct is, in the present case, unexpectedly easier than that of the usually observed host–guest binary complex. We tentatively suggest that the gaseous three-component $[G + Hs + Mx + H]^+$ supramolecular systems can achieve extra stabilization with respect to the related binary $[G + Mx + H]^+$ complexes by three different and synergistic factors: (i) an increased capability of delocalizing the positive charge, which, while mandatory for any MS process, can unfavourably affect the weak host–guest interactions; (ii) the internal energy excess, which promotes the spontaneous unimolecular dissociation of the host–guest binding, occurring in the

absence of collisional activation, can be distributed among a larger number of oscillators; (iii) the possibility that an Mx molecule chelates with both Hs and G by forming hydrogen bonds, thus establishing a bridge between them. Even with the less likely hypothesis of a covalent bond between Mx and G within the $[G + Hs + Mx + H]^+$ species, the stabilizing effects of the matrix molecule on the weak non-covalent Hs–G interaction should not be less important. Actually, while the energy excess and charge distribution capabilities remain almost the same as mentioned above, the bridge between Hs and G could be made by covalent Mx–G chemical bonding and Mx–Hs hydrogen bonding.

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