# Protonated neurotransmitters in the gas phase: a photochemical production method

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## Introduction

Under typical physiological conditions the majority of amines, including a variety of biomolecules, exist predominantly in a charged, protonated form. Until recently, gas-phase studies of biomolecules were limited to neutral species due, principally, to the difficulties of producing sufficient number densities of charged species at temperatures low enough to allow detailed spectroscopic examination. A new, photochemical, scheme which allows the selective protonation of biomolecules in sufficient quantities to avoid the need for ion traps[1)] is outlined in Figure 1.

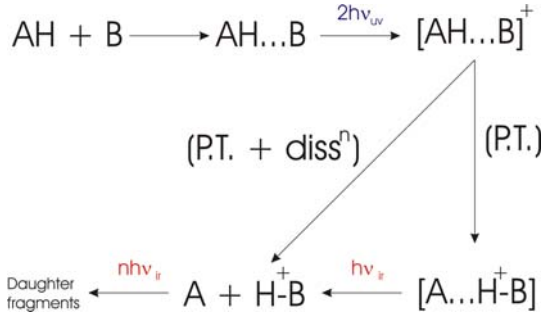


**Figure 1.** A new, photochemical production scheme for protonated biomolecules in the gas-phase.

Complexes between a proton donor (AH, e.g. phenol or naphthol) and an amine (B) are produced in a supersonic expansion and are ionised by 2-photon ionisation of AH. Efficient, exothermic proton transfer (P.T.) in the cation produces a complex between the protonated amine and a radical; the excess energy may be sufficient to dissociate the complex. Infra-red dissociation of the complex (single photon) or the bare species (requiring several photons) provides a spectral fingerprint for comparison with high-level quantum chemical computation.

Time-of-flight mass spectra and infra-red photodissociation spectra of cationic complexes between phenol and imidazole are shown in Figure 2. Mass peaks due to the cationic complex $(P\text{-}Imid)^+$ and protonated imidazole ($Imid\text{-}H^+$) are observed. The presence of complexes of phenol (phenol-water and phenol dimer) allows accurate mass calibration.

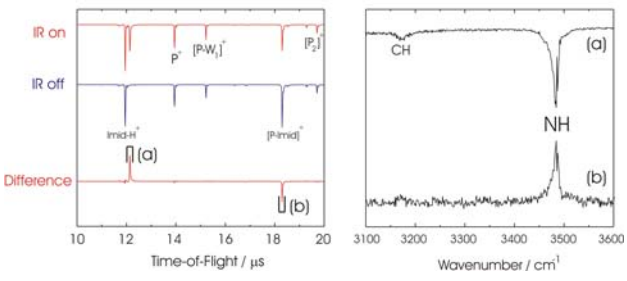


**Figure 2.** Time-of-flight mass spectra and infra-red photodissociation spectra of the cationic complexes between phenol and imidazole.

Absorption of infra-red radiation by the cationic complex ($[P\text{-}Imid]^+$) at 3483 $cm^{-1}$, and subsequent evaporation of the phenoxy radical, provides a spectral fingerprint of protonated imidazole. A significant improvement in signal to noise is achieved by *delaying* the infra-red laser relative to the UV laser by several hundred nanoseconds; during this interval, the ions begin to move towards the detector. Absorption of infra-red radiation, and subsequent dissociation, produces a fragment ion at a *different point of the extraction region* of the time-of-flight mass spectrometer resulting in a different arrival time at the detector relative to the fragment produced by UV alone. This "offset" allows photodissociation spectra to be recorded against a (near) zero background; this can be clearly seen in Figure 2 where the spectrum recorded in the fragment channel (denoted a) has a signal to noise ratio far superior to that measured by depletion of the parent ion (b).

The advantages of the scheme are selectivity (only species more basic than the phenol cation are protonated), sensitivity (mass spectrometric detection) and efficiency (sufficient ions are produced in a single laser shot to allow high-quality spectra to be recorded). In contrast to other production schemes (e.g. electrospray ionisation) mass filters and ion traps are *not* required.

## Protonated Neurotransmitters

Molecules based on the ethanolamine template (see Figure 3) constitute a significant portion of modern pharmaceuticals including treatments for high blood pressure, asthma etc.

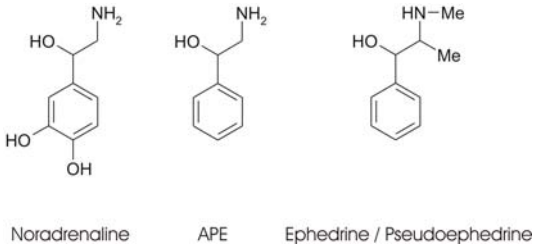


**Figure 3.** Neurotransmitters based on the ethanolamine template.

Infra-red photodissociation spectra of the cationic complexes between phenol and 2-amino-1-phenylethanol (APE) and the diastereoisomers ephedrine and pseudoephedrine are shown in Figure 4. Each spectrum displays features consistent with a proton-transferred structure; in particular the band at 3660 $cm^{-1}$ due to a non-hydrogen bonded OH group (c.f. ethanol) provides an excellent marker of protonation (the apparent structure on these bands is due to a reduction in infra-red power caused by absorption of atmospheric water). Additional bands due to the NH stretching modes of the protonated amine are observed in the region 3200-3400 $cm^{-1}$. The width of the bands (ca. 15-40 $cm^{-1}$ FWHM) reflects the substantial internal energy of the complex ions resulting from the 2-photon ionisation process and the exothermic proton transfer reaction. Calculations (MP2/6-311++G** single point at B3LYP/6-31+G* optimised geometries) indicate binding energies of the order of 100 $kJmol^{-1}$ (equivalent to 2.5 infra-red photons). The linear dependence of photodissociation on laser

power indicates a single photon absorption process; the proton transfer reaction alone is estimated to release some 57 kJmol$^{-1}$.

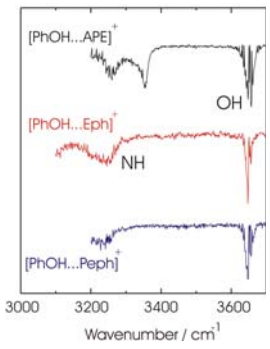


**Figure 4.** Infra-red photodissociation spectra of the cationic complexes between phenol and APE, and the diastereoisomers ephedrine and pseudoephedrine.

Computed (B3LYP/6-31+G*) structures of protonated APE and its complexes with the phenoxy radical are shown in Figure 5. The protonated species both feature an intramolecular hydrogen bond between the functional groups of the side-chain in which the amino group acts as the proton donor. In contrast, the neutral species have a reversed hydrogen bond in which the hydroxyl group is the proton donor[2]. Relative energies (computed using MP/6-311++G** at the DFT optimised geometries) favour the folded (GG) structure over the extended (AG) geometry; a folded geometry allows hydrogen bonding between the amino group and the π-system of the aromatic ring. Protonation greatly enhances such a bond; the folded and extended structures for the neutral species are essentially isoenergetic[2].

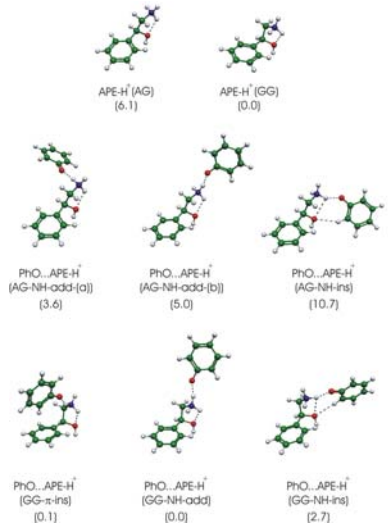


**Figure 5.** Computed structures (B3LYP/6-31+G*) of protonated APE and its complexes with the phenoxy radical. Relative energies (in kJmol$^{-1}$) are from MP2/6-311++G** single point calculations.

Complexation with the phenoxy radical causes only minimal changes in the geometry of the protonated species (similar effects are found for ephedrine and pseudoephedrine) although it does lead to an expansion in the number of possible structures. The preferred mode of binding is via the protonated amine group which forms a strong hydrogen bond with the oxygen of the phenoxy radical ($NH^+ \rightarrow$ OPh). Three such structures are found for each geometry of the protonated species; in general, the relative energies of the folded and extended ions are unchanged although one extended structure (AG-NH-add-(a)) is stabilised to a greater degree.

Computed and experimental infra-red spectra of the cationic complex of phenol and APE are shown in Figure 6 for the energetically favourable structures of bare and complexed APE-H$^+$. Good agreement is found between experiment and computation for all three complexes although a more definitive assignment is speculative; the non- (or weakly hydrogen bonded) NH stretches of the two energetically favourable complexes are different enough to allow a tentative assignment to both structures. The band at 3353 cm$^{-1}$ is assigned to the GG-π-ins structure while the shoulder to the blue of this feature can be assigned to the GG-NH-add complex. Contributions from other complexes (including structures with an extended arrangement of the protonated species) cannot be excluded.

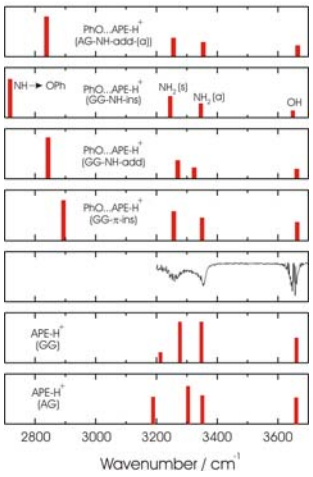


**Figure 6.** Experimental and computed (B3LYP/6-31+G*) infra-red spectrum of protonated APE and its complexes with the phenoxy radical. The intensity of the lowest frequency mode ($NH^+ \rightarrow$ OPh) has been scaled by a factor of 10 for clarity.

**Conclusion and future work.**

A new, photochemically based production scheme for the creation of protonated biomolecules in the gas phase has been demonstrated. Proton transfer from the phenol cation to a suitable amine group provides a selective and efficient source of protonated molecules. Spectral identification via infra-red photodissociation generates spectra for comparison with high level quantum chemical computation. Future work will address the problem of high internal energy through alternative proton donors (e.g. Naphthol) and creation of the protonated species in the collision region of a supersonic expansion.

**Acknowledgements.**

The authors wish to thank the Leverhulme Trust for financial support and the Lasers for Science Facility of the Rutherford Appleton Laboratory for the loan of the UV laser system used in this work.

**References**

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