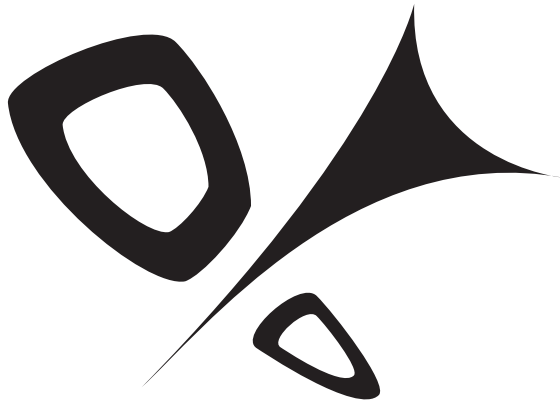


On the stability of molecules and microorganisms in interstellar and planetary environments



Zan Peeters

On the stability of molecules and microorganisms in interstellar and planetary environments

Proefschrift

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*Aristoteles vacuum definit: quod sit locus
corpore non repletus, aptus tamen repleri*

Aristotle defines vacuum: each place that is
not filled with a body, is filled anyway

Otto von Guericke, 1672

Vacuum sucks

Zan, 2007

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One

Introduction

Abstract

Space is filled with billions of stars, which are clustered in larger structures, named galaxies. Beyond our own galaxy (called Milky Way), there are billions of other galaxies. The space between the stars (the interstellar medium, ISM) and between the galaxies (intergalactic medium, IGM) was long thought to be vacuum. While these media are indeed relatively empty compared to a more familiar medium like the Earth's atmosphere, they do contain a certain level of matter in the form of gas and tiny solid particles. When atoms are present, chemistry can take place and molecules can be formed.

Organic molecules consist primarily of carbon and hydrogen atoms, but may also contain other atoms like oxygen and nitrogen. Organic molecules can grow up to very large sizes and may also form supramolecular structures. On Earth, all living organisms—from the simplest bacteria to complex mammals including human beings—are built from a collection of small organic molecules including amino acids, nucleotides, sugars, fatty acids, that can assemble into larger polymers such as proteins, RNA, and DNA, and large complexes (e.g. membranes). These building blocks need to be available on a planet before life can emerge. The central question in the origin of life is where organic chemistry started and how organic molecules assembled into self-organizing systems. It is likely that a combination of terrestrial (e.g. hydrothermal vents) and extraterrestrial sources (comets and meteorites) provided the first building blocks for life on the young Earth.

This introduction gives an overview of the organic chemistry that proceeds in interstellar, interplanetary, and planetary environments. During their life cycle, organic molecules formed in space may encounter degrading environmental conditions, such as ultraviolet (UV) radiation, cosmic rays, shocks, and elevated temperatures. The following chapters in this thesis investigate the stability of a variety of organic molecules and a salt-loving microorganism in interstellar and planetary environments.

1.1 Organics in space

Origin of the elements

The field of chemistry had its coming of age as a scientific discipline with the compilation of the periodic table by Mendeleev in 1869. Later, astronomers created their own version of the periodic table, aptly named *the astronomer's periodic table*, see figure 1.1. Here, the elements are sorted based on their universal abundance,

rather than their mass or atomic number. The elemental composition of the Universe is primarily based on the nucleosynthesis during the Big Bang. Between 100 and 300 seconds after the Big Bang, all the hydrogen and most of the helium in the universe, and small traces of deuterium, tritium, lithium, and beryllium were formed (see [Schramm \(1998\)](#) for an overview). All elements heavier than beryllium were formed later by nucleosynthesis during stellar evolution, probably starting as early as 300–500 My after the Big

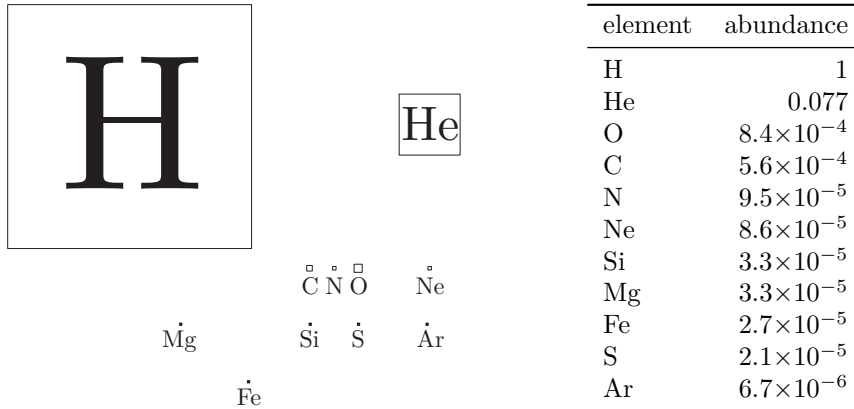


Figure 1.1

The astronomers' periodic table (left). The sizes of the boxes represent the relative cosmic abundances of the elements. The table (right) gives the cosmic abundances by number of atoms relative to hydrogen for those elements.

Bang ([Stark et al. 2007](#)).

Star formation

Stars are formed when a cloud of gas and dust contracts due to its own gravity. Heat generated by compression of the gas is initially dissipated in the infrared, mainly through emission of rotational lines by molecules such as CO. In this stage cloud contraction proceeds isothermally. When the density is high enough to trap the cooling radiation (at $\sim 10^7 \text{ cm}^{-3}$), the cloud core starts to heat up. When the core temperature reaches 10^7 K , nuclear fusion of hydrogen (^1H) to helium (^4He) is initiated. The formation of helium is a highly exothermic reaction and the heat produced by this reaction keeps the star from further

gravitational collapse. The star then reaches a temporary equilibrium, the duration of which is determined by the star's mass. During that period the star is called a main-sequence star. The Sun is an example of a star in its main sequence. For a recent overview of star formation, see [Lada \(2005\)](#).

When the hydrogen that fuels nuclear fusion reactions in the stellar core is consumed (approximately 10 Gyr for a star with the mass of the Sun), the pressure balance is no longer maintained and the star starts to gravitationally contract once more. While the core of the star contracts the outer layers expand, causing the star to increase in size. The compression of the core causes the temperature to rise further. When the

core temperature reaches 10^8 K, helium starts to fuse, forming carbon (^{12}C) and oxygen (^{16}O). Stars in this phase of their existence are called asymptotic giant branch (AGB) stars.

Circumstellar envelopes

Elemental carbon and oxygen are ejected from AGB stars by solar winds (Willson 2000). At a distance $>10^{13}$ cm from the central star, the temperature of the outflows has dropped enough for the carbon and oxygen atoms to form molecular bonds. C and O react quickly to form CO, which continues to be formed until one of the two elements is depleted. Early in the AGB phase of the star, O is the more abundant element and all C will be locked in CO. During that phase, the AGB produces O-rich silicate dust particles. Later, more C is dredged up from the star's core and when $\text{C/O} > 1$, oxygen will be depleted due to CO formation. The remaining C then forms molecules such as C_2H_2 and HCN. These molecules can engage in a rich gas-phase chemistry, initiated by shock waves from stellar pulsations. An important reaction that takes place is the polymerization of acetylene to benzene and larger polycyclic aromatic hydrocarbons (PAHs) (Frenklach & Feigelson 1989, Cherchneff et al. 1992). Benzene was tentatively detected in CRL618 by Cernicharo et al. (2001). These reactions take place in the region from 4×10^{13} – 10^{14} cm. Figure 1.2 shows the temperature profile through the circumstellar envelope of a typical AGB star. The plot is divided in five regions, each displaying a distinct type of chemistry as described above (adapted from Millar 2003).

Upon further migration outward from the star (10^{14} – 10^{15} cm) and subsequent further cooling, PAHs and other molecules coagulate to form small amorphous dust particles. These dust particles attenuate the UV light from the star on the inside and the interstellar UV radiation on the outside. In the region 10^{15} – 10^{16} cm, these dust grains create a high visual extinction that lowers the photodissociation rates and this zone is considered chemically quiet. Beyond 10^{16} cm the dust is diluted and the visual extinction A_V drops, allowing photodissociation and photoionization of molecules by the interstellar UV field to occur. This leads to a photon-driven type of chemistry and a wealth of reactions.

The carbon-rich envelope of IRC+ 10216, the prototypical example of an high mass-loss carbon star, contains a large variety of organic molecules (Glassgold 1996) and is dominated by the presence of carbon molecules, such as polyacetylenes (e.g. C_8H , Cernicharo & Guélin 1996), cyanopolynes (e.g. the largest uniquely identified interstellar molecule: HC_{11}N , Bell et al. 1982), sulfuretted chains (e.g. C_2S , C_3S , C_5S , Cernicharo et al. 1987, Bell et al. 1993), structural isomers (HCCNC vs. HCCCN , Gensheimer 1997), and the recently detected carbon-chain anions (C_6H^- and C_4H^- , McCarthy et al. 2006, Cernicharo et al. 2007). These molecules are formed in the inner parts of the circumstellar envelope, where the interstellar UV field is attenuated by the dust. Once these molecules reach the outer regions of the envelope, the outflowing gas is photodissociated and photoionized by the interstellar UV field to produce ions and radicals. Except for the most photochemically stable

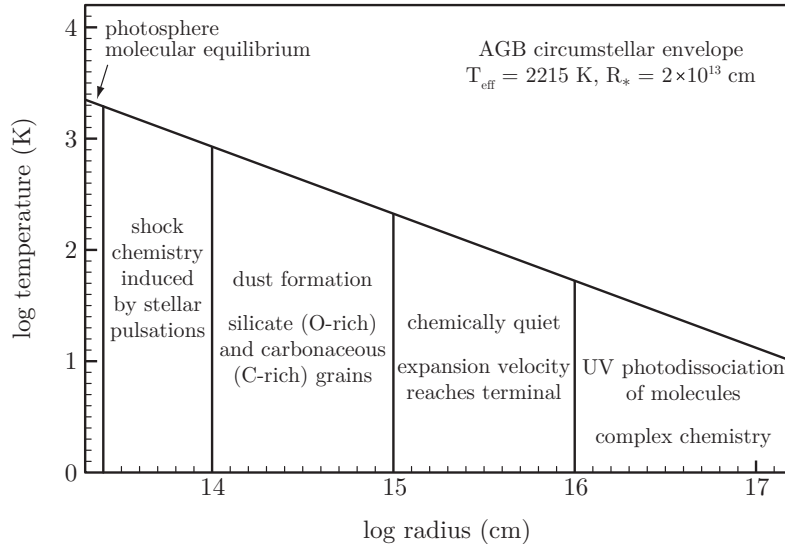


Figure 1.2

Temperature profile of a circumstellar envelope surrounding an AGB star with an effective temperature (T_{eff}) of 2215 K and a radius (R_*) of 2×10^{13} cm. The plot is divided into five regions, each having a distinct type of chemistry. Adapted from Millar (2003).

species (PAHs, dust grains), molecules formed in the circumstellar envelopes are destroyed in the outermost layers as they leave the circumstellar envelope and enter the diffuse ISM.

Near the end of the AGB phase, the star begins to pulsate. The pulses blow away its outer shells and the circumstellar envelope, thereby enriching the surrounding medium with the elements, molecules and dust grains it produced. The expanding cloud of gas and dust is called a protoplanetary nebula, and later (when the tem-

perature of the central star rises above 30,000 K) a planetary nebula. When the AGB star has converted all its helium into carbon and oxygen, the central star collapses into a white dwarf. For stars that started out with a mass >8 solar masses, nucleosynthesis continues to produce heavier elements up to ^{56}Fe . High mass stars end their lifetime in a violent supernova explosion.

Interstellar medium

The interstellar medium (ISM) comprises many different environments, with distinct physical properties. These environments can be divided into five main categories: the hot ionized medium, the warm ionized medium, the warm neutral medium, the atomic cold neutral medium, and the molecular cold neutral medium (Spitzer 1985). These categories are based on differences in temperature, hydrogen density, and the ionization state of hydrogen. A summary of these properties for each of the ISM components is given in table 1.1. A comprehensive overview of the composition and evolution of interstellar clouds is given by Wooden et al. (2004). Of the five categories listed in table 1.1, the warm neutral medium ($T \sim 10^4$ K, $n_{\text{H}} \sim 0.1 \text{ cm}^{-3}$, neutral H) is generally regarded as the intercloud medium. Outside areas where gravity dominates, the Milky Way is roughly in pressure equilibrium, with an average pressure (P/k) of $\sim 10^4 \text{ K cm}^{-3}$ (Field et al. 1969, McKee & Ostriker 1977).

Diffuse clouds

Diffuse clouds (atomic cold neutral medium) often surround denser and colder regions (Heiles 1967) and have filamentary or sheet-like structures. The main component of diffuse clouds is neutral atomic hydrogen (H) at densities ranging from $10\text{--}300 \text{ cm}^{-3}$. The temperature varies in the range $50\text{--}200$ K. Due to a low visual extinction ($A_V \sim 1$ magnitude), UV photons can easily penetrate the cloud to photodissociate and photoionize molecules. The molecules found in diffuse clouds are therefore usually simple species

such as CH^+ , CH, HCO^+ , HCN, HNC, CS, H_2CO , CO, OH, and C_2 (Lucas & Liszt 1997), or large UV-resilient molecules such as polycyclic aromatic hydrocarbons (PAHs) or other carbon clusters (Ehrenfreund & Charnley 2000). UV radiation in those clouds is dictated by the interstellar UV field as described by Draine (1978) that integrates to $\sim 10^8 \text{ photons cm}^{-2} \text{ s}^{-1}$ for the wavelength range $100\text{--}200 \text{ nm}$.

Molecular clouds

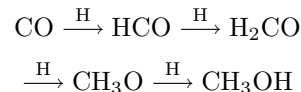
Molecular clouds are dense, cold regions of the ISM with hydrogen densities (H_2) in the range $10^3\text{--}10^4 \text{ cm}^{-3}$. The temperature in a molecular cloud is low, generally between $10\text{--}50$ K. Molecular clouds can contain many substructures, some of which are referred to as dense cores or clumps. These cores have a higher density than the surrounding cloud, ranging $10^4\text{--}10^6 \text{ cm}^{-3}$. The temperature of dense cores generally does not rise above 10 K. The visual extinction is usually very high and can reach up to hundreds of magnitudes. Because of this high extinction, photons from the interstellar UV field can not penetrate the cloud — hence its moniker dark cloud. Despite the high extinction, the UV field inside a dense core is not zero. Cosmic rays can penetrate the cloud and ionize hydrogen. Secondary electrons excite the surrounding hydrogen, which produces a low level of UV photons upon de-excitation (Prasad & Tarafdar 1983). This yields a UV flux of $\sim 10^3 \text{ photons cm}^{-2} \text{ s}^{-1}$ in the wavelength range $100\text{--}200 \text{ nm}$, with two main bands at 120 and 160 nm (Gredel et al. 1989). The low UV flux allows a large number of molecules to exist in dense clouds. A comprehen-

Table 1.1Phases of the interstellar medium (adapted from [Wooden et al. 2004](#)).

ISM component	common designation	T (K)	density (cm ⁻³)	state of hydrogen
hot ionized medium	coronal gas	10 ⁶	0.003	H ⁺
warm ionized medium	diffuse ionized gas	10 ⁴	>10	H ⁺
warm neutral medium	intercloud H I	10 ⁴	0.1	H
atomic cold neutral medium	diffuse clouds	100	10–100	H + H ₂
molecular cold neutral medium	molecular clouds	<50	10 ³ –10 ⁴	H ₂
	dense cores	10	10 ⁴ –10 ⁶	H ₂
hot molecular cores	protostellar cores	100–300	>10 ⁶	H ₂

sive and up-to-date list of all molecules detected in interstellar space (not only in dense clouds) can be found at <http://www.astrochemistry.net>. At the time of this writing, the counter for detected molecules rests at 151 unique molecules, or 231 including isotopomers. Of the 151 molecules currently identified, the majority is detected in dense clouds.

Chemistry in dense cores is dominated by solid-phase reactions on grain surfaces. Due to the low temperature in the cloud, sticking coefficients are close to unity and most molecules are condensed on dust grains. Consequently, these dust grains are covered by icy mantles. Methanol, for example, is formed efficiently in the solid phase in dense cores. In regions where the temperature is below 25 K, CO is depleted onto dust grains and successive additions of hydrogen atoms leads to the formation of CH₃OH ([Charnley 1997](#)).



When the temperature rises above 140 K (e.g. in hot molecular cores), methanol is evaporated into the gas phase. Once in the gas phase, protonated methanol can react to form dimethyl ether (DME, CH₃OCO₃) via methyl cation transfer ([Karpas & Mautner 1989](#)),



followed by electron dissociative recombination to yield CH₃OCH₃. Both cosmic rays and the cosmic ray induced UV field can deposit energy into the ice mantles, but the UV field is about 10 times more efficient than cosmic rays ([Shen et al. 2004](#)). UV photons can create radicals in the ice mantles. This has two consequences. First, UV-induced radical chemistry in the mantle can create molecules that may not have formed in the

gas-phase. Secondly, the energy released by the radicals causes the grain to heat and molecules to desorb into the gas-phase (Shen et al. 2004).

A number of molecules in interstellar ices have been detected in the infrared spectrum of dense clouds towards massive protostars (Gibb et al. 2004). The most abundant molecules in these ices are H_2O , CO , CO_2 , CH_3OH , and NH_3 (Whittet et al. 1996). Other species including OCS , H_2CO , HCOOH , CH_4 , and OCN^- have been observed towards massive protostars (d’Hendecourt et al. 1996) and low-mass protostars (Boogert et al. 2004). In laboratory simulations many other species have been produced by radiative processing of similar ices. The products that have been formed include radicals, new molecules, and complex organic materials (Gerakines et al. 2000).

Hot molecular cores

Stars are formed in the interiors of dense clouds. As described in section 1.1, stars form through the collapse of a dense cloud. This might be triggered by an energetic event such as a nearby supernova that generates shockwaves. These shockwaves create regions with higher local density, which act as the nucleation points for gravitational collapse. Such higher-density regions are also known as *hot (molecular) cores*, or *hot corinos* in the case of low-mass protostellar objects (Ceccarelli 2004). Hot molecular cores have temperatures in the range of 100–300 K and densities well over 10^6 cm^{-3} (table 1.1). Their lifetime is rather short, in the order of 10^5 years. Due to the higher temperature, the molecules that were originally condensed on the dust grains, evapo-

rate into the gas-phase. In the warm gas-phase, reactions can take place that form a wealth of new complex molecules (see e.g. Charnley et al. 1992).

Table 1.2 shows a list of molecules with their abundances that have been detected in a dense cloud (L134N), in protostellar ices (NGC 7538:IRS9), a protostellar hot core (Orion KL), in a low-mass protostellar hot corino (IRAS 16293-2422), the cooler and less dense outer part of IRAS 16293-2422, and in a cometary coma (Hale-Bopp) (adapted from Schöier et al. 2002). A remarkable recent detection is the first interstellar amino acid glycine towards the hot molecular cores Sgr B2(N-LMH), Orion KL, and W51 e1/e2 (Kuan & Charnley 2003), although the results are debated (Snyder et al. 2005, Cunningham et al. 2007).

1.2 Organics in the solar system

Solar system formation

During the collapse of a dense cloud into a protostellar object, not all gas and dust is incorporated into the central object. The slowly rotating presolar nebula shrinks in size and because of the conservation of angular momentum the rotation speed increases. As a result, the presolar nebula flattens into a disk. In the rotating protoplanetary disk, dust grains collide and stick together electrostatically (Blum 2000). This causes the grains to gradually grow in size which, in turn, increases the chance for collisions. The gas molecu-

Table 1.2

Selected molecules detected in the gas of a molecular cloud (L134N), protostellar ices (NGC 7538:IRS9), protostellar hot core (Orion KL), protostellar hot corino of IRAS 16293-2422 (I16293 HC), surrounding colder, less dense outer part of IRAS 16293-2422 (I16293 OP), and in a cometary coma (Hale-Bopp). Adapted from [Schöier et al. \(2002\)](#).

molecule	L134N	NGC7538:IRS9	Orion KL	I16293 HC	I16293 OP	Hale-Bopp
CO	1×10^{-4}	$(1-5) \times 10^{-6}$	1×10^{-4}	—	4.0×10^{-5}	1×10^{-5}
HCN	7×10^{-9}	$< 2 \times 10^{-6}$	4×10^{-7}	—	1.1×10^{-9}	1×10^{-7}
HC ₃ N	4×10^{-10}	—	2×10^{-9}	1.0×10^{-9}	$< 1.0 \times 10^{-10}$	1×10^{-8}
CH ₃ CN	$< 4 \times 10^{-10}$	—	2×10^{-8}	7.5×10^{-9}	$< 8.0 \times 10^{-11}$	1×10^{-8}
H ₂ CO	2×10^{-8}	$(1-4) \times 10^{-6}$	1×10^{-8}	6.0×10^{-8}	7.0×10^{-10}	5×10^{-7}
CH ₃ OH	5×10^{-9}	$(2-10) \times 10^{-6}$	2×10^{-7}	3.0×10^{-7}	3.5×10^{-10}	1×10^{-6}
CH ₃ OCH ₃	—	—	1×10^{-8}	2.4×10^{-7a}	—	—
HCOOH	3×10^{-10}	$(2-10) \times 10^{-7}$	8×10^{-10}	6.2×10^{-8a}	—	5×10^{-8}
HCOOCH ₃	—	—	1×10^{-8}	$< 6.0 \times 10^{-8}$	—	4×10^{-6}

^a from [Cazaux et al. \(2003\)](#)

les and dust grains in the disk start to coagulate and form larger particles. When the bodies reach kilometre size, gravitational interactions become dominant in the process ([Safronov & Zvjagina 1969](#)). The solid bodies (now called planetesimals) continue to grow by accretion, but gravitational pull can attract gas, dust, and other bodies over a longer range. When the planetesimals reach a size where gravitational pressure and heat from radioactive decay in the center causes the core to melt, the body is referred to as protoplanet. These protoplanets collide violently to form the terrestrial planets and the cores of the gas giants. [Lissauer \(1993, 2005\)](#) and [Goldreich et al. \(2004\)](#) give excellent overviews of the process of planet formation in the solar system.

The chemistry that started in the hot molecular cores, evolves during the presolar nebula and

protoplanetary disk phases. With the formation of a disk a range of new interactions influence the molecular composition and distribution in the protostellar region. The central young stellar object heats the disk from the inside, evaporating the grain-mantle ices and driving gas-phase chemistry. Further outward from the central young stellar object, a high dust abundance attenuates the light from the central object and keeps the temperature low. This allows molecules such as H₂O, CO, CO₂, NH₃, and CH₄ to condense onto cold dust grains. Cosmic rays can penetrate deep into the disk and may produce ions and radicals, which drive ion-molecule and neutral-neutral reactions in the outer regions of the disk. For a recent overview of the chemistry in protoplanetary disks, see [Bergin et al. \(2006\)](#). Turbulent mixing of the disk can dis-

tribute material throughout the disk. An important result from the recently returned Stardust mission, which collected dust particles from the tail of comet 81P/Wild2, is that mixing in the solar nebula occurred on a large scale and much more efficient than models previously predicted (Brownlee et al. 2006).

Planets

The International Astronomical Union recently adopted a new definition for planets and other solar system bodies (IAU 2006). The following three criteria were formulated:

1. a body has an orbit around the Sun,
2. a body has sufficient mass to assume hydrostatic equilibrium shape (nearly round), and
3. a body has cleared the neighbourhood around its orbit.

A *planet* is a body that fulfils all three of the above criteria, while a *dwarf planet* complies only with criterion 1 and 2 and, additionally, is not a satellite. All other objects that are in orbit around the Sun and are not satellites, will be referred to as *small solar system bodies*.

The planets can be divided into two groups: the terrestrial (or rocky) planets (Mercury, Venus, Earth, and Mars) and the Jovian planets (or gas giants, with Jupiter, Saturn, Uranus, and Neptune). Pluto, formerly the ninth planet, is now the prototype of the new class of dwarf planets. The division between the terrestrial planets and the gas giants relies upon the location

of the snowline, the distance from the Sun beyond which the temperature is low enough for a volatile molecule to condense. For water in the solar system this transition occurs around 3 AU. During the formation of the outer planets, dust grains in the protoplanetary disk beyond the snowline retained an ice mantle, causing the planetesimals in this region to grow faster and taking up a larger part of the material in the disk. In their present form, the gas giants are characterized by a mass that is high enough to gravitationally trap even the lightest gas, H_2 (between $15 M_\oplus$ for Uranus and $318 M_\oplus$ for Jupiter, where $M_\oplus$ is the mass of the Earth, 6.0×10^{24} kg). These planets do not have a distinct surface, but a gradually increasing density of gas, which becomes a supercritical fluid above the critical temperature and pressure.

The terrestrial planets have much lower masses than the gas giants (between $0.055 M_\oplus$ for Mercury and $1 M_\oplus$ for Earth). The composition of the terrestrial planets' atmospheres depends on the mass of the planet and the surface temperature. On Earth, N_2 , O_2 , and H_2O are stable in the atmosphere, while on Mars these compounds have escaped and CO_2 is the major atmospheric component.

1.3 Mars

Known as *Nergal* in Babylonian astronomy, as *Mangala* in Hindu mythology, as *Hr dš* (Horus the Red) to the ancient Egyptians (see textbox 1), as **מֵאָדִים** (Ma'adim, the one who blushes) in Hebrew, as *Ἄρης ἀστὴρ* (Ares' star) by the Greeks, the planet is in modern times better

known as the *Red Planet*, or simply *Mars*. Mars is a close neighbour to Earth, both in distance and in other properties¹. Because of the resemblance and proximity to Earth, Mars has been the subject of many past and ongoing space missions that search for clues of extinct or extant life.

Water on Mars

Images returned from Mars by the Mars Global Surveyor show the presence of gullies, features which may be explained by ground water seepage and surface run off (Malin & Edgett 2000). Malin et al. (2006) have also found gullies that formed in the past decade, by comparing images of the same area from 1999 and 2006, which suggests that, at least occasionally, liquid water may flow on Mars in the present day. The water that formed these gullies might come from large subsurface ice deposits, detected by the gamma-ray spectrometer on board the Mars Odyssey mission (Feldman et al. 2002). Cementation and bleaching along joints in layered deposits have been discovered in observations from the Mars Reconnaissance Orbiter (Okubo & McEwen 2007), indicating fluid alterations in the geological past by subsurface flows.

Martian mineralogy

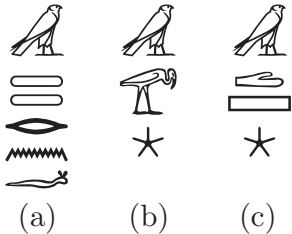
Data returned from the Observatoire pour la Mineralogie, l'Eau, les Glaces et l'Activité (OMEGA) spectrometer on Mars Express, the

Thermal Emission Spectrometer (TES) on Mars Global Surveyor, the Thermal Emission Imaging System (THEMIS), and the Mössbauer spectrometers on the Mars Exploration Rovers have provided many new insights into the planet's mineralogy. The main mineral on the surface of Mars are the silicates olivine, pyroxene, and plagioclase (Bibring et al. 2005). Clay minerals including Fe-rich smectites (such as nontronite), Fe/Mg-phyllosilicates (e.g. chamosite), and Al-rich phyllosilicates (such as montmorillonite) have also been identified (Poulet et al. 2005). These clays are mainly localised on the south Noachian crust. Iron oxide minerals have long been considered to be present on Mars due to the typical red colour of the surface. Iron oxides have been found in various forms of hematite (Morris et al. 1997) and goethite (Kirkland & Herr 2000). Sulfate minerals have been discovered as jarosite (Klingelhöfer et al. 2004), gypsum (Langevin et al. 2005) and kieserite (Wang et al. 2006). Jarosite, hematite, and several other minerals found on Mars, are usually formed in the presence of water. This notion, along with the above mentioned gullies, indicate that there was probably liquid water on Mars for a geologically relevant period. Chevrier & Mathé (2007) give an comprehensive overview of the mineralogical and geological results from the recent Mars missions.

Based on the impact cratering records of the martian surface Hartmann & Neukum (2001) have derived absolute dates for the relative peri-

¹Actually, Venus is closer in distance, 0.72 AU compared to 1.52 AU for Mars. Also Venus' mass ($0.81 M_{\oplus}$) is a closer match to Earth than Mars ($0.11 M_{\oplus}$), but with an average surface temperature of 750 K and an atmosphere of 86 bar CO₂ Venus is inhospitable to Earth life.

Textbox 1: Mars in ancient Egypt



In Egyptian mythology stars and planets were thought to represent gods. In the case of Mars, this was the god Horus (Hr, pronounced as *Her*). The symbol of Horus was the falcon, which is the first character in each of the hieroglyphs depicted here. The names used to point out Mars changed over time. The oldest of the three hieroglyphs shown here (a) was in use during the New Kingdom and the third Intermediate Period (around 1539–715

BC). It can be translated as ‘Horachte is his name’, where Horachte means ‘Horus of the horizon’. The second hieroglyph (b) is usually transliterated as Hr dšr and pronounced as *Her desjer*. This name was in common use during the Greco-Roman period (332 BC – 395 AD) and is translated ‘Horus the Red’. Hieroglyph (c) is an alternative spelling of (b) and is transliterated as Hr dš. For an extensive overview of the names given by the ancient Egyptians to astronomical objects, see [Neugebauer & Parker \(1969\)](#).

ods in the martian geological history defined by [Tanaka \(1986\)](#). [Bibring et al. \(2006\)](#) overlaid the impact cratering timeline with a timeline based on mineralogical data. They define three periods: the phyllosian (early, characterized by the presence of phyllosilicates, found on Noachian terrains), the theiikian (mid period, characterized by the presence of sulfates, linked with Hesperian terrain), and the siderikian (mid to late, characterized by the presence of iron oxides, overlaps with Amazonian period). The different periods are represented in figure 1.3. During the phyllosian, phyllosilicates were formed, which indicates the presence of liquid water during that period. This suggests a warmer and wetter climate

in the past. In the interval between the phyllosian and the theiikian, there was probably a period of global change which included high volcanic activity. Large amounts of sulphides were released by the volcanic activity, which acidified the water. As the water gradually evaporated during the theiikian, sulfate minerals were formed. When water evaporation completed, the dry and oxidizing siderikian period started ([Bibring et al. 2006](#)).

Organics on Mars

Based on the recent discoveries concerning the mineralogy and geology of Mars (see previous

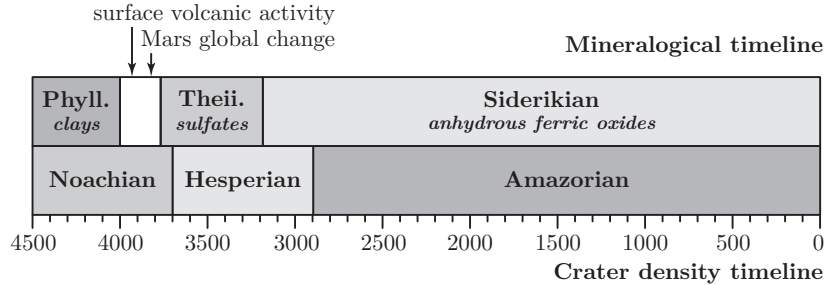


Figure 1.3

The major epochs in Mars' geological history, based on mineralogical data (top) and cratering data (bottom). Phyll. stands for Phyllosian and Theii. is short for Theiikian. Adapted from [Bibring et al. \(2006\)](#), cratering chronology from [Hartmann et al. \(2001\)](#).

section), the current view holds that Mars was warmer and wetter in the past. If there was indeed a prolonged period of liquid water on the surface of Mars, the possibility exists that life emerged. One of the goals of the 1976 Viking Landers was to find life on Mars. The Labeled Release (LR) experiment mixed an aqueous growth medium containing ^{14}C -labelled organic compounds with collected martian soil samples and monitored the head space for release of $^{14}\text{CO}_2$. The Gas Exchange (GEx) experiment also introduced an aqueous growth medium to soil samples, but monitored the head space for any change in gas composition. A gas-chromatograph-mass-spectrometer (GCMS) measured the volatile organic content of the soil samples, as they were heated to 200, 350, or 500 $^{\circ}\text{C}$. The LR and GEx experiments both showed a rapid release of $^{14}\text{CO}_2$ (LR) and O_2 and CO_2 (GEx), but the gas evolution quickly ceased and

did not recover when new medium was added. Also, the GCMS did not detect any organic molecules above the detection limit of several parts per billion ([Biemann et al. 1976, 1977](#)). These results lead to the conclusion that the Mars soil is probably not biologically active and that chemical reactivity of the martian soil caused the positive responses in the LR and GEx experiments ([Klein 1979](#)).

Although the Viking GCMS may have missed certain types of molecules ([Benner et al. 2000, Glavin et al. 2001](#)), the non-detection of organics is still surprising because some organic molecules were expected to be present as a result of meteoritic influx ([Flynn 1996](#)). The discrepancy between expected and detected levels of organics is usually attributed to degradation. Solar UV photolysis of atmospheric gas molecules, and subsequent recombination, can produce oxidizing species in the atmosphere, such

as H, OH, HO₂, H₂O₂, O, and O₃ (see e.g. [Nair et al. 1994](#)). These species are relatively stable in the martian atmosphere and can diffuse into the soil, where they may oxidize organic compounds ([Hunten 1979](#)). H₂O₂ was detected in the martian atmosphere by [Encrenaz et al. \(2004\)](#). Besides an atmospheric origin, oxidants may also be produced by interaction of UV radiation with minerals ([Quinn & Zent 1999](#), [Yen et al. 2000](#)). Another relatively unexplored source of degradation is cosmic rays. While UV radiation is attenuated by the soil within a few millimetres, energetic solar protons and cosmic rays have a much greater penetration depth. [Dartnell et al. \(2007\)](#) have shown that cultures of *E. coli*, *B. subtilis*, and *D. radiodurans* at a depth of 2 m in the martian soil would be inactivated by cosmic rays within 30,000, 250,000, and 450,000 years, respectively. MeV protons can destroy organic molecules like benzene ([Ruiterkamp et al. 2005](#)), although larger PAH molecules are altered rather than destroyed by fast protons ([Bernstein et al. 2003](#)).

1.4 Thesis outline

The previous sections explained the formation and evolution of organic molecules in different interstellar and planetary environments. A prerequisite for the formation of molecules is the presence of elements. The elements are formed in the early universe and during nucleosynthesis in stars. From those elements, large organic molecules are most efficiently formed in cold dense regions in the interstellar medium. Collapsing dense interstellar clouds provide the raw mate-

rial for star and planetary formation.

Tiny interstellar dust particles grow to large kilometre-sized planetesimals in the protoplanetary disk. These planetesimals collide to form planets. Not all the material of the protoplanetary disk is incorporated into the planets during planetary formation. Some material remains in the planetary system as small solar system bodies, grouped into comets and asteroids. In the early history of our solar system, these small bodies frequently impacted the young planets. By this process, comets and asteroids delivered their molecular inventory to the young planetary surfaces.

Liquid water seems to be essential for all biological systems on Earth. On a planet where liquid water is present, organic molecules may engage in a wide range of reactions. Prebiotic chemistry on Earth involving terrestrial and extraterrestrial matter, probably led to complex structures with emerging functions that subsequently formed the first living cells. Organic compounds in interstellar and planetary environments follow an evolutionary cycle and may be exposed to harsh environments during their lifetime. Investigating the stability of these compounds may provide insight in the link that connects the chemistry in interstellar space and the origin of life of Earth.

Dimethyl ether (DME) is one of the largest organic molecules detected in interstellar space. It is found in regions of high-mass star formation, but only in the hot cores where it exists in the gas-phase. In **chapter 2** we present laboratory data on the UV photolysis rate of DME. These data were extrapolated to survival times

for relevant interstellar regions. The photolysis rates were also included in chemical models that calculate the evolution of a hot core. We found that DME is rapidly destroyed by UV radiation in the diffuse interstellar medium and the solar system at 1 AU. Furthermore, we found that UV photolysis of DME in hot molecular cores has a negligible effect on its chemical evolution.

Nitrogen-containing cyclic organic molecules (N-heterocycles) play an important role in terrestrial biology. For example, the nucleobases of the genetic material are N-heterocycles. In **chapter 3**, we report the destruction rates for the N-heterocycles pyridine, pyrimidine, and s-triazine, measured in the laboratory in a simulated interstellar environment. The results show that the photostability of the N-heterocycles decreases with an increasing number of nitrogen atoms in the ring (figure 1.4). All three N-heterocycles are rapidly destroyed in the diffuse interstellar medium and solar system environments. In dense clouds, pyridine and pyrimidine but not s-triazine, are stable for a period that is comparable to the average lifetime of a cloud ($\sim 10^6$ year). We discuss that the formation of N-heterocycles in the gas-phase follows a similar pathway as the formation of benzene, but that it is unlikely to produce molecules with more than 1 nitrogen in the ring. Several classes of N-heterocycles have been found in meteorites (carbonaceous chondrites), but stable isotope data are needed to determine the extraterrestrial origin of these molecules.

Nucleobases are the carriers of genetic information in all living cells. Extraterrestrial nucleobases have been detected only in carbonaceous

meteorites. **Chapter 4** reports on the UV photostability of the nucleobases adenine and uracil. We found that adenine and uracil are rapidly destroyed in the diffuse interstellar medium and the solar system at 1 AU, but both are stable in a dense cloud for at least the lifetime of the cloud. We discuss possible formation routes and conclude that nucleobases will be difficult to form outside the solar system. In the solar system they are likely synthesized on the parent bodies of meteorites or on comets, where they are protected from solar UV photons.

Mars is thought to receive 2.4×10^5 kg of carbon year⁻¹ by meteoritic influx. However, the Viking missions did not detect any organic molecules above a detection limit of a few ppb in the martian surface. This discrepancy is usually attributed to oxidizing reactions in the martian soil. In **chapter 5** we report on experiments that tested the stability of amino acids in Mars soil analogues exposed to a simulated martian environment. Mars soil analogues obtained from the Atacama desert were found to vary strongly in pH, ion concentrations, and redox potential, even when the samples were obtained only a few metres apart. The stability of embedded amino acids in the Atacama soil were compared with an iron-rich deposit from Denmark and a sample of the Orgueil meteorite. The differences in amino acid stability are attributed to a different mineral composition of the Mars soil analogues. We conclude that clay minerals can stabilize amino acids and protect them against destruction.

Halophilic archaea have the capability to survive in desiccated and high salt environments. In **chapter 6** we tested the survival of halophilic

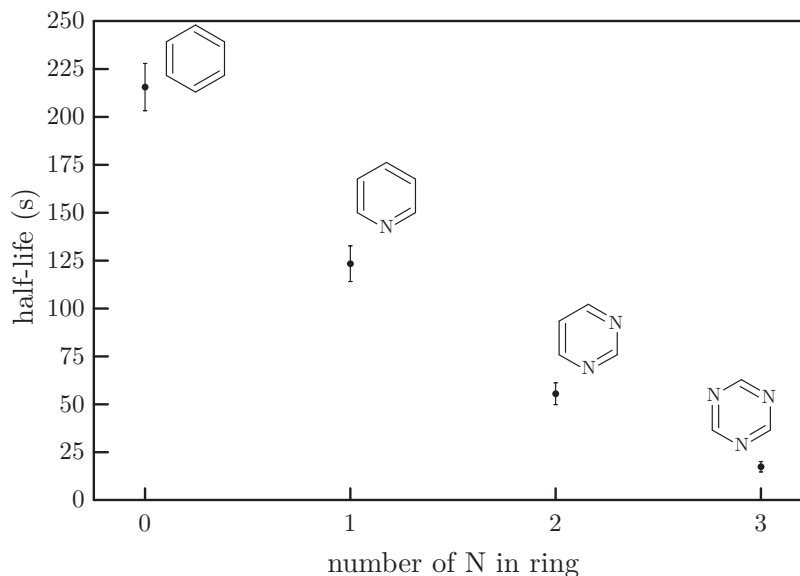


Figure 1.4

The UV photolysis half-life measured in the laboratory for four six-ring molecules containing 0 (benzene), 1 (pyridine), 2 (pyrimidine), or 3 (s-triazine) nitrogen atoms in the ring.

archaeon *Natronorubrum* sp. strain HG-1, when subjected to high temperature (70 °C), low temperature (4 and −20 °C), desiccation, and irradiation with wavelengths >200 nm. We also desiccated cells mixed with Atacama desert soil. The results show that *Natronorubrum* sp. strain HG-1 is not affected by storage at 4 or −20 °C, while storage at 70 °C completely inhibited growth. Desiccated samples were fully recovered upon rehydration, but desiccated samples mixed with Atacama soil did not show any growth. We conclude that *Natronorubrum* sp. strain HG-1 is

not likely to survive on Mars. Additionally, *Natronorubrum* sp. HG-1 could not have survived the early Earth’s ocean, due to its vulnerability to high temperatures.

The origin of prebiotic molecules on Earth is currently unknown, but it is likely that both terrestrial and extraterrestrial sources contributed to the organic inventory on the early Earth. The research described in this thesis focuses on the stability of small organic molecules. The results show that amino acids, nucleobases, and their precursors are very fragile under most interstel-

lar conditions. Amino acids are also degraded by environmental conditions on Mars. Certain types of minerals may shield these compounds from degradation.

The formation of some prebiotic molecules, e.g. nucleobases, is unlikely to proceed efficiently in interstellar environments. The combination of a high formation reaction barrier and fast degradation by UV radiation, make it unlikely that those organic molecules are present when the planetary system is formed. Many organic molecules, including amino acids and nucleobases, have been found in meteorites, indicating that these molecules can be synthesized in the solar system on the meteoritic parent bodies.

In order to contribute to the organic inventory of a newly formed planet, prebiotic molecules must have formed in protected environments in the solar system, such as comets, meteorites, planetary oceans, or subsurface regions. Given the right conditions on the planet, this starting material may react to form larger structures and, ultimately, life.

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Two

Astrochemistry of dimethyl ether

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Abstract

Dimethyl ether (DME, CH_3OCH_3) is one of the largest organic molecules detected in the interstellar gas and shows high abundances in star-forming regions, known as hot molecular cores. The observed DME might be formed on grains or by secondary gas phase reactions from a precursor molecule, which in turn was sublimed into the gas phase from the grain surface. Studies on the stability and degradation pathways of DME therefore provide important constraints on the evolutionary cycle of large organic molecules and chemical pathways in the interstellar medium. We studied the UV photodestruction rate of DME in a solid argon matrix. DME was destroyed with a half-life of 54 seconds under laboratory conditions, which corresponds to 8.3×10^5 years in a dense cloud. We discuss the UV photochemistry of DME in the context of two issues: its formation mechanism and its chemistry in hot cores. Chemical models of shielded hot core regions indicate that UV photodestruction is relatively unimportant for DME, even by the internally-generated radiation field. These models clearly show that gas phase processes are almost certainly responsible for the formation of interstellar DME.

2.1 Introduction

In the energetic environments of forming massive and low-mass protostars, so-called hot molecular cores, thermal heating or sputtering in shock waves can liberate the molecular ice mantles that cover interstellar dust grains (e.g. [van Dishoeck & Blake 1998](#), [Ehrenfreund & Charnley 2000](#)). Observations of these regions can therefore be used to probe the nature of grain-surface chemistry. The desorbed molecules can initiate distinctive gas phase reaction pathways, that can lead to the growth of highly complex organic molecules ([Charnley et al. 1995](#)), oxygen-nitrogen molecular differentiation ([Charnley et al. 1992](#), [Caselli et al. 1993](#)), and the possibility of comparing dynamical time-scales through ‘chemical clocks’ ([Charnley 1997b](#), [Hatchell et al. 1998](#)).

Dimethyl ether (DME, CH_3OCH_3) is ubiquitous in regions of massive star formation where

it is found to exist solely in hot cores (e.g. [Turner 1991](#), [Sutton et al. 1995](#), [Nummelin et al. 2000](#)). It has also been detected recently in the hot core (viz. the ‘hot corino’) of the low-mass binary system IRAS 16293-2422 ([Cazaux et al. 2003](#), [Kuan et al. 2004](#)). Observations of comet Hale-Bopp have failed to detect DME in the coma ([Crovisier et al. 2004](#)) and it is also undetected in cold molecular clouds (e.g. [Friberg et al. 1988](#)).

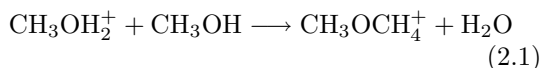
We are currently undertaking a study on the photostability of complex molecules, to assess their survivability in different astronomical environments and delineate possible formation mechanisms ([Ehrenfreund et al. 2001a,b](#), [Peeters et al. 2003, 2005](#)). As a part of this program, we have now extended our previous experimental work to consider the photochemistry of DME ([Schriver-Mazzuoli et al. 2002](#), [Schriver et al. 2004](#)). Infrared (IR) spectroscopic studies of DME have been

performed previously in the laboratory in argon and ice matrices (Schriver-Mazzuoli et al. 2002, Schriver et al. 2004). In those experiments we determined IR spectroscopic features and photoproducts upon irradiation with UV. In this paper we will put those measurements into astrophysical context using quantitative measurements concerning the UV stability of DME in the gas phase.

In the following sections we will describe the experiments and results of UV photolysis experiments of DME in a solid argon matrix. As a test of the putative grain-surface production of DME in star-forming regions, IR spectra of DME/H₂O mixtures will be compared to recent spectra of those regions. Finally, the astronomical application of laboratory and theoretical studies to the role of DME chemistry in hot cores will be reconsidered.

The origin of DME

DME was proposed by Blake et al. (1987) to originate in the gas phase. Methyl cation transfer to methanol and formaldehyde leads to DME and methyl formate (HCOOCH₃), respectively, after electron dissociative recombinations. The reaction

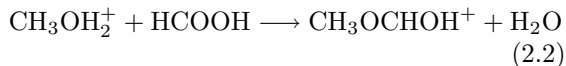


is well-studied in the laboratory with a measured rate coefficient (Karpas & Mautner 1989). Its inclusion in chemical models where methanol molecules are evaporated from grain surfaces can explain the high abundances of DME and methyl formate ($\sim 10^{-8}$ – 10^{-7}), as well as the

CH₃OCH₃/CH₃OH abundance ratios of ~ 0.02 – 0.1 %, that are typically observed (Charnley et al. 1992, Caselli et al. 1993).

However, observations of the G34.3 and W3(H₂O) cores appear to indicate that their methanol abundances are lower than in other cores and comparable with those of its putative daughter molecules (MacDonald et al. 1996, Helmich & van Dishoeck 1997). As models predict that at most a few per cent of the evaporated methanol is converted to DME and methyl formate (Charnley et al. 1992), it would be difficult to reproduce the DME abundance in G34.3 even with the higher methanol abundance determined by Mehringer & Snyder (1996). There are also problems in interpreting the DME and methyl formate abundances of low-mass ‘hot corinos’ within the standard picture. Observations of HCOOCH₃ and CH₃OCH₃ in IRAS 16293-2422 led Cazaux et al. (2003) to derive CH₃OCH₃/CH₃OH and HCOOCH₃/CH₃OH ratios close to unity. Cazaux et al. (2003) concluded that the IRAS 16293-2422 core is not old enough for post-evaporation gas phase chemistry to have formed these molecules and hence that, like methanol, they are the products of grain-surface reactions.

The proposed synthesis of HCOOCH₃ in the gas phase is unlikely (Charnley 1997a), since experiments demonstrate that methyl cation transfer to H₂CO does not occur (Karpas & Mautner 1989, see also Horn et al. 2004). An alternative pathway to HCOOCH₃ has been suggested (Charnley 1997a), involving the abundant mantle molecule HCOOH (Schutte et al. 1996) instead of H₂CO, see equation 2.2.



A surface origin for HCOOCH_3 is possible since this molecule is observed in comets (e.g. [Bockelée-Morvan et al. 2004](#)) and coma chemistry calculations show that it must emanate from the nuclear ices ([Rodgers & Charnley 2001b](#)). An important question is therefore whether there is sufficient time, post-evaporation, for hot core chemistry to synthesise the observed abundances of HCOOCH_3 , or whether it too must be formed on grains?

The build-up of a molecule as complex as DME is unlikely to proceed efficiently on cold grain surfaces by addition reactions beginning from single atoms (e.g. [Charnley 1997a](#)). Hydrogen atom additions to an unsaturated precursor molecule, as occurs for example in the formation of methanol in CO ices ([Watanabe & Kouchi 2002](#)), is also debatable. The known candidates containing a C-O-C structure ([Ikeda et al. 2001](#)) are more likely to lead to other stable products before six hydrogens are present, for example the reduction of ethylene oxide to oxirane ([Charnley 2001](#)). As any alternative precursors would require major structural rearrangements, DME may be assembled on grains by the association of simple radical functional groups, for example between methoxy and methyl radicals (e.g. [Allen & Robinson 1977](#), [Sorrell 2001](#), [Hollis & Churchwell 2001](#)). These radicals would have to be produced from abundant pre-existing molecules (i.e. methanol and methane) and so a source of energy is necessary, both to break chemical bonds and to provide mobility to the products. Irradia-

tion of ices, either by heavy cosmic ray particles or by UV photons, has been studied extensively in the laboratory (e.g. [Allamandola et al. 1997](#), [Gerakines et al. 2001](#), [Ehrenfreund et al. 2001b](#)). In fact, experiments involving UV photolysis and warming of interstellar ice mixtures have shown that the IR signatures of methyl ethers can be produced ([Bernstein et al. 1995](#)). Although the identification of individual compounds has not been made, it is very likely that DME is amongst them. Thus, experiments show that photolysis and photostability could both play a role in the solid state production of DME. In this paper, we investigate this further based on our previous experimental work.

DME in hot molecular cores

Whether or not DME is formed solely on grains or in gas phase reactions can be important for understanding its chemical evolution in hot cores (and ‘hot corinos’). The utility of gaseous DME as a ‘chemical clock’ for the post-evaporation phase lies in the fact that it is only formed from methanol, whose abundance in both the solid and gaseous phases can be measured. This means that the evolution of the $\text{CH}_3\text{OCH}_3/\text{CH}_3\text{OH}$ abundance ratio in hot cores depends mainly on the cosmic-ray ionization rate and the density, and less so on the temperature. Model calculations of DME formed in ion-molecule chemistry, typically suggest post-evaporation ages of a few times 10^4 years for hot cores, based on the time-scale where the DME abundance peaks ([Charnley et al. 1992](#)).

However, if DME is formed solely on grains

by some unknown process, its gas phase kinetics will be markedly different. In the gas phase, DME is destroyed primarily in reactions with H^+ , He^+ and C^+ and by protonation by H_3^+ , HCO^+ , H_3O^+ and CH_3OH_2^+ . In electron recombinations, protonated DME can also recombine to methanol, leading to a net loss (Le Teuff et al. 2000). Neglect of DME gas phase formation through reaction 2.1 therefore leads to kinetics where the evaporated DME abundance suffers an exponential decline over the age of the core. Thus, in this case higher DME abundances can be expected to occur much earlier in the hot core lifetime.

Although hot molecular cores have high visual extinctions and thus effectively shield against the interstellar UV field, the photochemistry of gaseous DME can still become an issue in determining its evolution, irrespective of the actual formation mechanism. At the high densities of these regions ($\sim 10^6\text{--}10^8\text{ cm}^{-3}$), simple estimates indicate that photodestruction by the weak flux of Prasad-Tarafdar UV photons (Prasad & Tarafdar 1983) can be competitive with destruction in the above ion-molecule reactions. Furthermore, the stellar UV radiation field will be important for the chemistry, either close to forming protostars (Stäuber et al. 2004), or near the end of the hot core phase when an ultracompact HII region starts to form (Kurtz et al. 2000).

2.2 Experimental

Matrix isolation spectroscopy and UV destruction of DME in an argon matrix was performed

on a standard matrix isolation set-up (for a detailed description of the set-up Hudgins et al. 1994, Peeters et al. 2003, see) with a background pressure of $\sim 10^{-9}$ mbar and a CsI sample window thermally connected to a closed-cycle helium cryostat, capable of cooling down to 12 K. The window and cryostat are rotatable without breaking the vacuum.

Argon (Praxair, 99.9990 %) and DME (Sigma-Aldrich, 99+ %) were lead through a cold trap cooled with a solid CO_2 /acetone slush (-80°C) to remove any trace of water and CO_2 from the gas, before mixing them in a glass gas mixing set-up with a background pressure of $\sim 10^{-6}$ mbar and fitted with a capacitance manometer (Leybold). DME was diluted in argon to a ratio of 1:750.

The argon/DME mixture was deposited onto the 12 K CsI substrate window to a thickness of $1\text{ }\mu\text{m}$, controlled by standard laser interferometry. As a control, the DME column density was checked from the IR spectrum using the band strengths found by McKean et al. (1996), confirming the mixing ratio to $\sim 10\%$ accuracy. A total layer thickness of $1\text{ }\mu\text{m}$ allowed for full penetration of UV light through the sample.

UV irradiation was performed using a microwave-excited hydrogen-flow lamp (Ophos) with a flux of $4.8 \times 10^{14}\text{ photons cm}^{-2}\text{ s}^{-1}$. The lamp was calibrated using the O_2 to O_3 conversion method (actinometry) described by Cottin et al. (2003). UV irradiation was performed in 10 second intervals to a total of 2 minutes of irradiation, followed by 1 minute intervals of irradiation until the DME sample was completely destroyed. After every UV irradiation interval,

in situ IR spectra in the range 4000–500 cm^{-1} were recorded on an Excalibur FTS-4000 Fourier-transform IR spectrometer (Bio-Rad) at 1 cm^{-1} resolution, see [Peeters et al. \(2003\)](#).

2.3 Results

UV photostability of DME

IR spectra of DME condensed at low temperatures as well as the spectra of DME in H_2O , in an argon matrix and in a nitrogen matrix, followed by UV photolysis, have been reported by [Schriver-Mazzuoli et al. \(2002\)](#) and [Schriver et al. \(2004\)](#). We have measured the UV photodestruction rate of DME isolated in an argon matrix at 12 K. In figure [2.1](#), IR spectra of DME after deposition (before UV irradiation) and after 10 seconds, 1 minute and 10 minutes of irradiation are shown. Relevant bands of DME are found between 2990–2820 cm^{-1} (many small bands) and at 1456 cm^{-1} (double peak, ν_{14} and ν_{19}), 1173 cm^{-1} (ν_{16}), 1098 cm^{-1} (ν_{17}) and 926 cm^{-1} (ν_6), see [McKean et al. \(1996\)](#).

Upon irradiation with UV new features arose in the spectrum. These new bands are listed in table [2.1](#). The photoproducts were identified and compared to the list of photoproducts reported by [Schriver et al. \(2004\)](#). The new bands found at 3020 and 1305 cm^{-1} are attributed to methane, which is in agreement with the identification by [Schriver et al. \(2004\)](#). However, in contrast to [Schriver et al. \(2004\)](#) we did not find formaldehyde (bands at 2864, 2797, 1741 and 1498 cm^{-1}), but instead a band appeared at 1863 cm^{-1} , which was assigned to the radical

HCO. [Schriver et al. \(2004\)](#) found this band only in the nitrogen matrix. Additionally, bands were detected at 2021 and 904 cm^{-1} , which were tentatively assigned to HCCO (ethynyloxy radical, [Jacox & Olson 1987](#)) and $[\text{HAr}_2]^+$ ([Milligan & Jacox 1973](#)), respectively. A third band found at 1234 cm^{-1} could not be identified, but is probably due to contamination of the vacuum set-up.

All bands of DME diminished upon irradiation with UV light. The rate of destruction was calculated by plotting the natural log of the relative integrated peak area of the strongest bands at 1173, 1098 and 926 cm^{-1} (see figure [2.1](#)) against irradiation time. The slope of a linear fit through these data points yielded the DME destruction rate, see table [2.2](#).

Spectroscopic properties of DME in ice matrices

The IR spectrum of DME trapped in water ice has been reported by [Schriver-Mazzuoli et al. \(2002\)](#) for different temperatures in the range 11–160 K. The authors showed that at low temperatures in a 1:10 mixed DME/ H_2O ice the strongest bands can be found at 1161 and 1086 cm^{-1} (8.613 and 9.208 μm). No changes occur when the samples are heated to 110 K. At 130 K these bands move to 1168 and 1096 cm^{-1} (8.562 and 9.124 μm), and their relative intensities change. Above 160 K, DME disappears from the sample, because a change in crystal structure of the water ice allows trapped volatile species to escape.

Figure [2.2](#) shows a comparison between the laboratory IR spectrum of a 10:1 H_2O /DME mixture (top) and the ISO SWS spectrum of

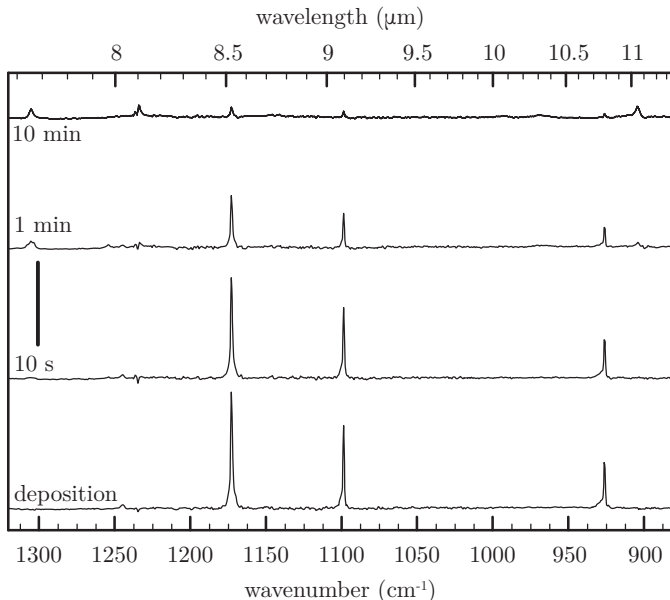


Figure 2.1

The IR spectrum of DME isolated in argon at 12 K after deposition and after 10 seconds, 1 minute and 10 minutes of UV irradiation. The strongest vibrational transitions of DME can be found in the 1300–900 cm^{-1} region displayed here. The vertical bar scales to 0.01 absorbance units.

high mass protostar W33A (bottom). The DME fundamental transitions are identified by vertical bands. The DME bands in the 3000 cm^{-1} (3.3 μm) region have low intrinsic band strengths. Additionally, they are mixed into the wing of the OH bending mode of H_2O that falls in the 3125–2778 cm^{-1} (3.2–3.6 μm) range.

The two DME peaks visible in the labo-

ratory spectrum at 1461 cm^{-1} (6.84 μm) and 1455 cm^{-1} (6.87 μm) are obscured in the W33A spectrum by the band at $\sim 1460 \text{ cm}^{-1}$ (6.85 μm), which was ascribed to the ν_4 transition of NH_4^+ (Schutte & Khanna 2003). The strongest peaks in the DME spectrum, 1173 cm^{-1} (8.53 μm), 1098 cm^{-1} (9.11 μm) and 926 cm^{-1} (10.8 μm), see section 2.3, are completely obscured

Table 2.1

Wavenumbers ($\tilde{\nu}$) of the photoproducts that appeared after UV irradiation of matrix isolated DME. *a* This work, *b* Schriver et al. (2004), *c* Jacox & Olson (1987), *d* not identified, *e* Milligan & Jacox (1973). The band found at 1863 cm^{-1} , assigned to the HCO radical, was also found by Schriver et al. (2004), but only in a nitrogen matrix.

$\tilde{\nu}^a$ (cm^{-1})	$\tilde{\nu}^b$ (cm^{-1})	assignment ^b
3020	3024	CH_4
	2864	H_2CO
	2797	H_2CO
2350	2345	CO_2
2140	2138	CO
2021		HCCO^c
1863	1860	HCO
	1741	H_2CO
	1498	H_2CO
1305	1305	CH_4
1234		— ^d
	1039	O_3
904		$[\text{HAr}_2]^+{}^e$

Table 2.2

Photon fluxes and DME destruction rates in the laboratory, the diffuse interstellar medium (ISM), a dense cloud, and the solar system at 1 AU from the Sun. φ_{tot} Is the total flux of photons ($\text{photons cm}^{-2} \text{ s}^{-1}$) at wavelengths where DME destruction is efficient, and k_{abs} and k_{destr} are the destruction rates (s^{-1}) calculated respectively from gas phase absorption spectra and our experimental results. τ Is the resulting half-life of DME in each region.

	φ_{tot} ($\text{cm}^{-2} \text{ s}^{-1}$)	k_{abs} (s^{-1})	k_{destr} (s^{-1})	τ
laboratory	4.3×10^{14}	3.1×10^{-3}	1.3×10^{-2}	54 s
diffuse ISM	1.4×10^8	1.3×10^{-9}	3.8×10^{-9}	5.8 yr
dense cloud	1×10^3	—	2.7×10^{-14}	0.82 Myr
solar system (1 AU)	6.7×10^{12}	2.9×10^{-5}	1.8×10^{-4}	64 min

by the strong silicate bands at 1000 cm^{-1} (10 μm). A comparison between the laboratory DME/H₂O IR spectrum and the ISO SWS spectrum of W33A predicts that it will be difficult to detect DME in the solid state. The DME bands are likely to be obscured by other stronger bands, from more abundant species. Furthermore, the laboratory spectrum was made with a DME/H₂O ratio of 10%, while a much lower DME/H₂O ratio is expected to be present in H₂O dominated ices (~ 0.02 – 0.2%).

2.4 Discussion

DME photodestruction in different astronomical environments

Photoabsorption cross sections for gas phase DME have been measured by numerous groups

(see for example figure 5 of [Feng et al. 2000](#)). These experiments showed that the cross section varies strongly with wavelength, peaking at $\sim 80 \text{ nm}$ ([Koizumi et al. 1986](#)). [Crovisier \(1994\)](#) reported a photodestruction rate in the solar system at 1 AU from a quiet sun of $3.11 \times 10^{-5} \text{ s}^{-1}$ (with a contribution of $6.6 \times 10^{-6} \text{ s}^{-1}$ due to Ly- α), based on the absorption data by [Suto et al. \(1988\)](#).

In the experiments described in section 2.2 we have measured the destruction rate of DME directly, upon irradiation with a hydrogen lamp. The spectrum of this lamp, as measured by [Cottin et al. \(2003\)](#) is shown in figure 2.3 (dashed line). The spectrum of [Cottin et al. \(2003\)](#) was scaled to yield an integrated flux of $4.8 \times 10^{14} \text{ photons cm}^{-2} \text{ s}^{-1}$, the value obtained by calibration of our lamp, also described by [Cottin et al. \(2003\)](#). The DME absorption spectrum as measured by [Suto et al. \(1988\)](#) is also shown in

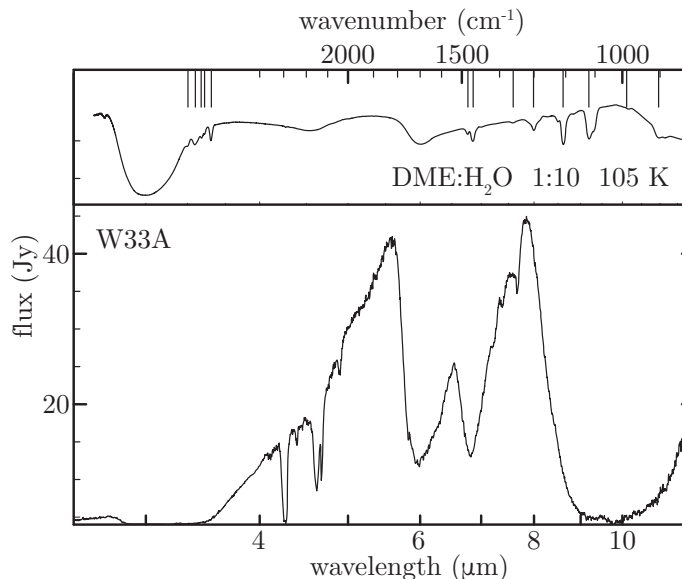


Figure 2.2

Comparison of a 1:10 DME/H₂O mixture at 105 K (top) with the ISO SWS spectrum of the high mass embedded protostar W33A (bottom). The vertical lines identify the DME bands.

figure 2.3 (solid line). The strongest emission line in the lamp spectrum, around 156–168 nm, overlaps with the $\tilde{D}$ transition of DME, which results in a high absorption rate. The absorption cross section of DME is much higher at 121 nm, but the Ly- α emission of the hydrogen lamp contains at most 5% of the total energy between 100–200 nm (Cottin et al. 2003).

Also shown in figure 2.3 are the spectrum of a quiet sun at 1 AU (dash-dotted line, ASTM

E490-AM0 spectrum¹) and the Draine field between 100–200 nm (dotted line, Draine 1978, Roberge et al. 1991). The solar spectrum has a larger Ly- α contribution to the total energy delivered between 100–200 nm ($\sim 9\%$) compared to the hydrogen lamp.

The spectra in figure 2.3 can be used to calculate the UV absorption rate of DME for the three different UV fields. The interval in which all four traces overlap lies between 119 and 195

¹from <http://redc.nrel.gov/solar/spectra/am0/>

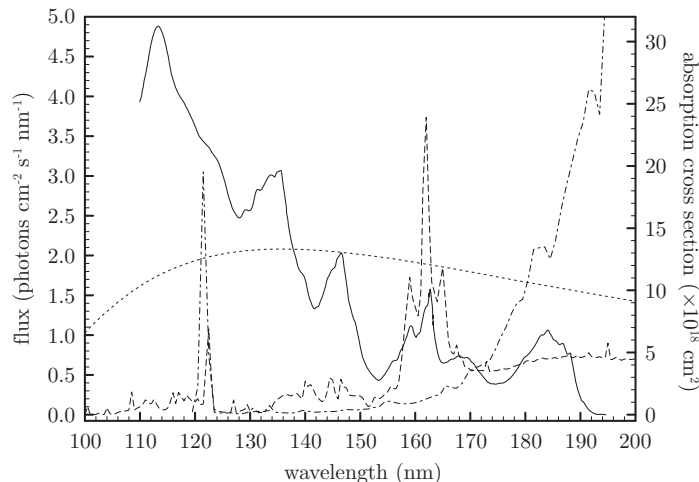


Figure 2.3

A comparison of different astronomical UV sources with the DME absorption spectrum (solid line) in the range 100–200 nm. The sources shown here are the laboratory hydrogen lamp (dashed line, [Cottin et al. \(2003\)](#)), the ASTM E490-AM0 solar spectrum (dash-dotted line), and the Draine interstellar field (dotted line, [Draine \(1978\)](#), [Roberge et al. \(1991\)](#)). The UV sources scale to the left Y-axis by a factor 10^{13} for the hydrogen lamp, 10^{11} for the solar spectrum and 10^6 for the Draine field. The DME absorption spectrum scales to the right Y-axis.

nm. We used these wavelengths as the integration limits for the following calculations. The UV absorption rate k_{abs} [s^{-1}] is given by:

$$k_{\text{abs}} = \int_{119}^{195} \sigma_{\text{abs}}(\lambda) \phi(\lambda) d\lambda \quad (2.3)$$

where $\sigma_{\text{abs}}(\lambda)$ is the absorption cross section [cm^2] and $\phi(\lambda)$ is the UV flux [$\text{photons cm}^{-2} \text{s}^{-1} \text{nm}^{-1}$] at wavelength λ [nm]. When the destruction rates are calculated in this way, we find values of $1.3 \times 10^{-9} \text{s}^{-1}$ for the Draine field in

the diffuse interstellar medium and $2.9 \times 10^{-5} \text{s}^{-1}$ for the solar system, see table 2.2. These numbers are comparable to those given by [Roberge et al. \(1991\)](#) and [Crovisier \(1994\)](#), respectively, although slightly lower. This can be explained by the fact that the integration here is done over the wavelength range 119–195 nm, while others have used the full range of data available. The calculation was also done for the hydrogen lamp and yielded a rate of $3.1 \times 10^{-3} \text{s}^{-1}$.

Our experimental data yield a destruction

rate of $1.3 \times 10^{-2} \text{ s}^{-1}$, which is larger than the expected theoretical value of $3.1 \times 10^{-3} \text{ s}^{-1}$. In order to extrapolate our results to the photodestruction rate of DME in space, we calculated the mean absorption cross section, $\bar{\sigma}$, of DME for photons with energies between 119–195 nm. From the DME absorption spectrum shown in figure 2.3 we derive $\bar{\sigma} = 2.7 \times 10^{-17} \text{ cm}^2$. The destruction rate of DME in different environments can then be approximated by

$$k_{\text{destr}} = \bar{\sigma} \phi_{\text{tot}} \quad (2.4)$$

where ϕ_{tot} is the integrated UV flux between 119 and 195 nm. Table 2.2 lists the derived destruction rates, as well as the rates calculated via integration of the absorption spectrum (k_{abs} , equation 2.3).

An important difference between k_{abs} and k_{destr} is that the former was measured in hot ($\sim 290 \text{ K}$) gas phase, while the latter was measured in a cold (12 K) argon matrix. At first glance, the destruction in the argon matrix would be expected to be *slower* than the gas phase, because the energy of the UV photons can be dissipated into the matrix. Also, because the reaction products are confined to a small space in the argon matrix, back-formation of the original molecules could occur. However, we find that the destruction in the matrix actually goes *faster* in the argon matrix. Contaminants in the matrix could enhance the destruction reaction, but we dismiss the idea of contaminants, because the most abundant and reactive contaminant, H_2O , is present in such low concentration, that it falls below the IR detection limit. This means that if water would be present in the matrix, the $\text{H}_2\text{O}/\text{DME}$

ratio would be < 0.02 .

The destruction rates are easily converted into half-lives, the time in which 50% of an initial amount of DME is destroyed. The half-life τ [s] is equal to $\ln 2 / k_{\text{destr}}$. In table 2.2 the half-life is also calculated for a dense cloud environment. In a dense cloud the UV flux is $1 \times 10^3 \text{ photons cm}^{-2} \text{ s}^{-1}$, according to Prasad & Tarafdar (1983). The UV field in this environment is believed to be comparable to the hydrogen lamp, albeit with a lower total flux, since both UV fields are generated by hydrogen emission. This means that the destruction rate in dark clouds can be found by scaling the destruction rate in the lab with the ratio of the integrated fluxes: $10^3 / 4.8 \times 10^{14}$. The resulting half-life of $8.2 \times 10^5 \text{ year}$ means that DME could survive the average lifetime ($\sim 10^6 \text{ year}$) of a dense cloud (Elmegreen 2000, Hartmann et al. 2001).

DME in hot molecular cores

In order to investigate whether DME is formed solely by reaction 2.1 or if a grain reaction is required, we have performed model calculations of hot core chemistry. The high visual extinction of hot cores (~ 300 magnitudes) means that photodestruction is only possible through the Prasad-Tarafdar radiation field. Thus, we also considered the effect of these photons in the post-evaporation chemistry.

We used our standard hot core model as described in Rodgers & Charnley (2001a), and assumed a temperature of 100 K, a density of $n(\text{H}_2) = 10^7 \text{ cm}^{-3}$, and a cosmic ray ionization rate, ζ , of $1.3 \times 10^{-17} \text{ s}^{-1}$. Our only alterations to

the reaction scheme were to turn off three radiative association reactions involving CH_3^+ : those with water, methanol, and acetaldehyde which form CH_3OH_2^+ , $(\text{CH}_3)_2\text{OH}^+$, and $\text{C}_3\text{H}_7\text{O}^+$, respectively. Initial abundances are as in [Rodgers & Charnley \(2001a\)](#), except for water which we injected with $n(\text{H}_2\text{O})/n(\text{H}_2) = 6 \times 10^{-5}$. The initial ammonia abundance was set to zero. In the Rodgers & Charnley model, DME is formed in the gas phase almost entirely via reaction 2.1, with a very small contribution due to the radiative association of CH_3^+ and methanol. DME is destroyed by protonation (it is assumed 50% of the dissociative recombinations of $(\text{CH}_3)_2\text{OH}^+$ lead back to $\text{DME} + \text{H}$, and 50 % result in the fragmentation to methanol and CH_3), and by reactions with He^+ , H^+ , C^+ , and CH_3^+ . In model runs with the Prasad-Tarafdar radiation field included, DME was also destroyed by photons, with a rate of $2833 \times \zeta$.

We computed models for three specific scenarios.

- (a) A reference hot core model similar to that of [Rodgers & Charnley \(2001a\)](#), as described above, both with and without the effects of the Prasad-Tarafdar radiation field. Ices were evaporated instantaneously and the subsequent chemistry followed.
- (b) As in the models of (a), except that reaction 2.1 was turned off and DME was instead injected from grains at an abundance of $n(\text{CH}_3\text{OCH}_3)/n(\text{H}_2) = 1 \times 10^{-7}$, consistent with observed upper limits in interstellar ices (see section 2.3).
- (c) As in the models of (b), except that the in-

jected methanol abundance was lowered to equal that of DME.

Figure 2.4 shows the chemical evolution in each scenario. From figure 2.4(a) it can be seen that Prasad-Tarafdar photons have little effect on the methanol-DME chemistry. This is because, due to the relatively large proton affinities of DME and methanol, the dominant ions in these models are $(\text{CH}_3)_2\text{OH}^+$ and CH_3OH_2^+ . The major loss route for methanol is in fact the self-methylation in reaction 2.1, whereas the protonation of DME, eventually formed from the recombination of $(\text{CH}_3)_2\text{OH}^+$, is mainly by CH_3OH_2^+ . Hence, CH_3OH_2^+ dominates both the production and loss of DME in hot cores. In the cases where reaction 2.1 was assumed not to occur, figures 2.4 (b) and (c) show that DME injection from dust leads to a different chemical evolution.

In these models the DME is again destroyed through protonation and the methanol abundance actually increases due to the fact that it is a product of one of the dissociative electron recombination channels of $(\text{CH}_3)_2\text{OH}^+$. In cases (b) and (c), Prasad-Tarafdar photons can affect the long-term destruction of methanol, however, even increasing the cosmic ray ionization rate to $5 \times 10^{-17} \text{ s}^{-1}$ (not shown) only produces marginally faster destruction of DME.

Injection of DME from dust leads to its peak abundance occurring much earlier in the evolution, and so would suggest much younger ages, of typically less than 10^4 years, than when it forms solely by gas phase chemistry (i.e. $\gtrsim 10^4$ years, see figure 2.4(a)). Only a model with an initially low methanol abundance can reproduce and maintain $\text{CH}_3\text{OCH}_3/\text{CH}_3\text{OH} \sim 1$. Based on

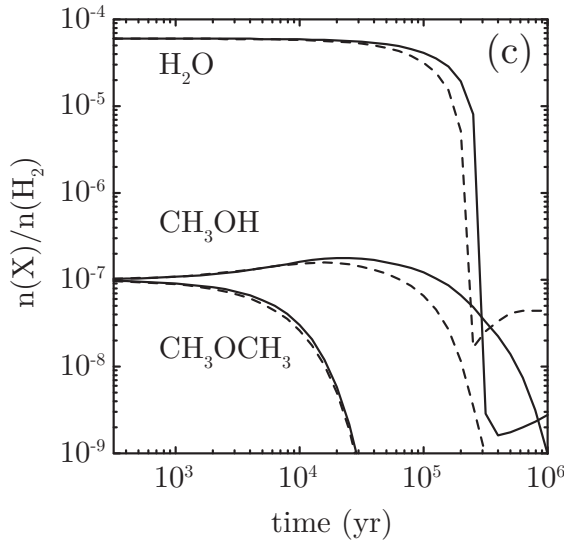
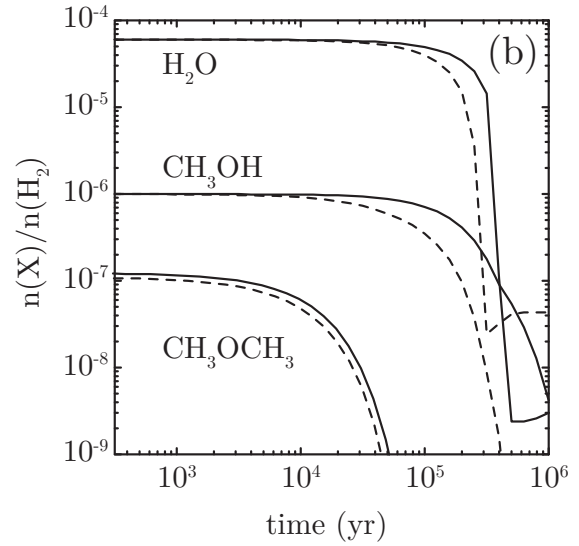
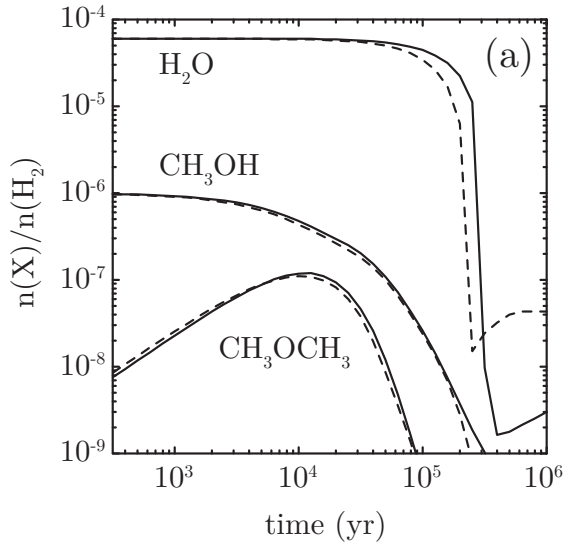


Figure 2.4

Hot core evolution of water, methanol and DME in three model scenarios. Broken curves are models which include photodestruction from the Prasad-Tarafdar radiation field. See text for details.

this ratio the maximum core ages would be less than a few thousand years. In a hot core rich in methanol, it is clear from figures 2.4 that DME production through reaction 2.1 can dominate any grain-surface contribution, unless they happen to be observed very early in their evolution.

Thus, one might ask if, in general, interstellar DME could come from both sources, where the grain-surface contribution only becomes evident in the presence of relatively methanol-poor ices? Unlike many proposed pathways to the formation of other complex interstellar molecules (e.g. Charnley 2001), reaction 2.1 is experimentally verified and, as shown in figure 2.4(a), can reproduce the observed DME abundances. Only three studies, based on single-dish observations, have thus far presented derived ratios of $\text{CH}_3\text{OCH}_3/\text{CH}_3\text{OH}\sim 1$ (MacDonald et al. 1996, Helmich & van Dishoeck 1997, Cazaux et al. 2003). Cazaux et al. (2003) determined that, when combined with data from Schöier et al. (2002), the abundance ratios of many O-containing molecules in IRAS 16293-2422 relative to methanol were almost all much higher than found elsewhere. In particular, Cazaux et al. (2003) argued that an observed ratio of $\text{HCOOCH}_3/\text{CH}_3\text{OH}\sim 1$ would rule out gas phase production, as would a similarly high ratio involving DME. Although grain-surface production of HCOOCH_3 is quite possible (see section 2.1), a serious problem for this interpretation of the origin of DME lies in the fact that Cazaux et al. (2003) formed their $\text{HCOOCH}_3/\text{CH}_3\text{OH}$ and $\text{CH}_3\text{OCH}_3/\text{CH}_3\text{OH}$ ratios using abundances from two different set of observations. Schöier et al. (2002) derived their methanol abundances

using a gas column density of $\text{N}(\text{H}_2) = 1.6\times 10^{24} \text{ cm}^{-2}$, whereas Cazaux et al. (2003) used $\text{N}(\text{H}_2) = 7.5\times 10^{22} \text{ cm}^{-2}$ in deriving their HCOOCH_3 and CH_3OCH_3 abundances. Interferometric observations show that IRAS 16293-2422 can be resolved into two so-called ‘hot corinos’ (Kuan et al. 2004, Bottinelli et al. 2004, Huang et al. 2005). In this case, abundances derived using more realistic (i.e. larger) values of $\text{N}(\text{H}_2)\gtrsim 10^{24} \text{ cm}^{-2}$ lead to HCOOCH_3 and CH_3OCH_3 abundances ($\sim 10^{-9}$ – 10^{-8}), and ratios relative to $\text{CH}_3\text{OH}\sim 0.01$ – 0.1 , that are more in accord with those predicted by theory (Kuan et al. 2004, Bottinelli et al. 2004, Huang et al. 2005).

2.5 Conclusions

We have considered the chemistry of DME in astronomical environments from experimental, observational and theoretical perspectives. We specifically addressed issues relating to the survivability of DME against UV destruction, its actual formation mechanism, and its chemistry around massive protostars.

- We investigated the UV photostability of DME in the laboratory. We compared our results for UV photodestruction of DME in argon matrices with previous work for DME in water ice matrices and in the gas phase. All bands of DME diminished upon irradiation with UV light. The major photoproducts which could be identified include methane, HCO and HCCO.
- We used our laboratory measurements to estimate the lifetime of gaseous DME un-

der various astronomical conditions. The half-life of DME in the diffuse interstellar medium corresponds to 5.8 years, in a dense cloud it corresponds to 0.8 Myr and in the solar system at 1 AU distance DME can only survive for 64 minutes. The low photostability of DME could be a reason why DME has not been detected in the cometary coma.

- We compared laboratory IR spectra of DME/water ice mixtures with the IR spectra of the astronomical ices surrounding a forming massive protostar W33A. We find that the strongest bands of DME are obscured by the dominant bands of water ice and silicates in interstellar spectra. The detection of interstellar solid DME is therefore a very difficult task.
- We have modelled the gas phase chemistry of DME in hot molecular cores. We find that inclusion of Prasad-Tarafdar photons has a negligible effect on its chemical evolution. The high efficiency of DME production by gas phase chemistry, coupled with its observed quite low upper limits in ices, suggest that grain-surface reactions are at most a minor source of interstellar DME.

Given the difficulty in detecting DME in interstellar ices, searches for deuterated isotopomers of DME in hot cores and setting more stringent upper limits on the abundance of DME in comets (cf. Crovisier et al. 2004) would appear to be most helpful for assessing the potential importance of a grain-surface contribution. However, applying Ockham’s Razor leads us to the

conclusion that gas phase synthesis via the self-methylation of methanol is the chemical pathway to DME in space.

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Three

Formation and photostability of N-heterocycles in space: the effect of nitrogen on the photostability of small aromatic molecules

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Abstract

Nitrogen-containing cyclic organic molecules (N-heterocycles) play important roles in terrestrial biology, for example as the nucleobases in genetic material. It has previously been shown that nucleobases are unlikely to form and survive in interstellar and circumstellar environments. Also, they were found to be unstable against ultraviolet (UV) radiation. However, nucleobases were detected in carbonaceous meteorites, suggesting their formation and survival is possible outside the Earth. In this study, the nucleobase precursor pyrimidine and the related N-heterocycles pyridine and s-triazine were tested for UV stability. All three N-heterocycles were found to photolyse rapidly and their stability decreased with an increasing number of nitrogen atoms in the ring. The laboratory results were extrapolated to astronomically relevant environments. In the diffuse interstellar medium (ISM) these N-heterocycles in the gas phase would be destroyed in 10–100 years, while in the Solar System at 1 AU distance from the Sun their lifetime would not extend beyond several hours. The only environment where small N-heterocycles could survive, is in dense clouds. Pyridine and pyrimidine, but not s-triazine, could survive the average lifetime of such a cloud. The regions of circumstellar envelopes where dust attenuates the UV flux, may provide a source for the detection of N-heterocycles. We conclude that these results have important consequences for the detectability of N-heterocycles in astronomical environments.

3.1 Introduction

Nucleobases are nitrogen-containing heterocyclic aromatic compounds (N-heterocycles), found in the genetic material of all living organisms on Earth. They can be divided into two groups according to their molecular structure: the purine derivatives (adenine, guanine, xanthine and hypoxanthine) and the pyrimidine derivatives (cytosine, thymine and uracil). The only source where extraterrestrial nucleobases have been detected so far, is in meteorites ([van der Velden & Schwartz 1977](#), [Stoks & Schwartz 1979, 1981](#)). [Peeters et al. \(2003\)](#) discussed possible formation routes for nucleobases in circumstellar environments, followed by an examination of the stability of these molecules against UV radiation. The

results showed that adenine and uracil are easily degraded, in a matter of years by the UV field present in the diffuse interstellar medium. Nucleobases are not likely to be formed in dense interstellar regions ([Smith et al. 2001](#)).

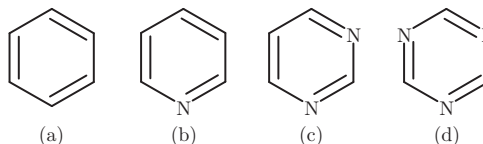


Figure 3.1

Four six-membered cyclic aromatic molecules: (a) benzene, (b) pyridine, (c) pyrimidine, and (d) 1,3,5-triazine (or s-triazine), having zero, one, two, and three nitrogen atoms per ring, respectively.

In the extended gas and dust envelopes of late-type stars, polymerisation of acetylene can produce benzene, larger polycyclic aromatic hydrocarbons (PAHs) and large carbonaceous dust particles (e.g. [Cherchneff et al. 1992](#)). N-heterocycles may form as by-products of acetylene polymerisation. Intermediate replacement of C_2H_2 by HCN could lead to direct incorporation of a nitrogen atom into a growing aromatic ring ([Ricca et al. 2001](#)), forming pyridine. Due to a small HCN/ C_2H_2 ratio in these envelopes, this scenario predicts decreasing abundances of aromatic molecules containing more than one nitrogen atom per ring, such as pyrimidine (two nitrogen atoms) and s-triazine (three nitrogen atoms), see figure 3.1.

Inclusion of nitrogen into the symmetric skeleton of aromatic hydrocarbon compounds induces a permanent dipole moment, which allows the molecule to be observed in a pure rotational spectrum. Pyridine has been the subject of more than 20 published studies on rotational spectroscopy. The millimetre wave rotational spectrum of the parent isotopomer was investigated up to $J = 60$ and 205 GHz by [Wlodarczak et al. \(1988\)](#) and accurate spectroscopic constants were reported. The rotational spectrum of pyrimidine has recently been thoroughly reinvestigated with the use of several different spectrometers ([Kisiel et al. 1999](#)) and precise spectroscopic constants of the ground state and several excited vibrational states, as well as the molecular geometry have been determined. s-Triazine is a symmetric top molecule with zero permanent dipole moment and no results from rotational spectroscopy are available.

Of the four molecules in figure 3.1 only benzene has been detected, by ISO observations in post-asymptotic giant branch (AGB) object CRL618 ([Cernicharo et al. 2001](#)). In parallel with the experimental work reported here and by [Peeters et al. \(2003\)](#), we have undertaken an observational program to search for various N-heterocycles in interstellar and circumstellar environments ([Kuan et al. 2003c, 2004](#)). Searches for pyridine towards carbon-rich AGBs did not yield any positive results. The upper limits of total molecular column densities that were derived for pyridine, are $N_{\text{tot}} < 7 \times 10^{12} \text{ cm}^{-2}$ in IRC+10216 and $2 \times 10^{13} \text{ cm}^{-2}$ in CRL618 ([Kuan et al. 2003a, 2004](#)). For nucleic acid building block pyrimidine upper limits of 1.7×10^{14} , 2.4×10^{14} and $3.4 \times 10^{14} \text{ cm}^{-2}$ were found in the objects Sgr B2(N), Orion KL and W51 e1/e2, respectively ([Kuan et al. 2003c](#)). Obviously, no searches have been conducted for s-triazine.

It is crucial to investigate the photostability of small N-heterocycles in order to understand their role in interstellar and circumstellar gas phase chemistry. Their destruction rates determine the lifetime and abundance of these molecules in interstellar regions and, consequently, their detectability. In the present paper, we measured the UV photostability of the nucleobase precursor molecule pyrimidine, along with the N-heterocycles pyridine and s-triazine, to investigate the effect of the number of nitrogen atoms in the ring on the photostability of these molecules. UV photolysis experiments on benzene have been reported by [Ruiterkamp et al. \(2005\)](#) and the results for this hydrocarbon are compared with the results for the nitrogen-containing het-

erocycles. In the following sections we will describe the photostability experiments and their results, and discuss the implications of these results for astronomical observations of small N-heterocycles in different space environments.

3.2 Experimental

Matrix isolation spectroscopy and UV destruction of pyridine, pyrimidine and s-triazine was performed on a standard matrix isolation set-up (Peeters et al. (2003), see also Hudgins et al. (1994) for a detailed description of the set-up) with a background pressure of $\sim 10^{-9}$ mbar and a CsI substrate window thermally connected to a closed-cycle helium cryostat, capable of cooling down to 12 K. The substrate window and cryostat can rotate without breaking the vacuum.

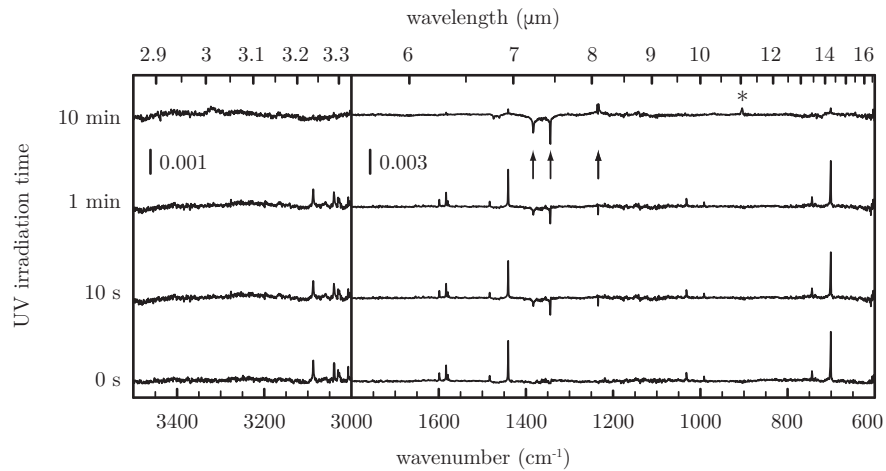
For the matrix material 99.999 % pure argon (Praxair) was used. Pyridine (99.8 %, Fluka) and pyrimidine (99 %, Aldrich) were prepared by four freeze-pump-thaw cycles to remove dissolved gases. The hygroscopic s-triazine (98 %, Merck) was handled exclusively under a nitrogen atmosphere in a glove-box, followed by four freeze-pump-thaw cycles. The vapour of each of these molecules was diluted in argon to a 1:750 ratio, using a glass gas-mixing line with a background pressure of $\sim 10^{-6}$ mbar. The absolute pressures of the gases were measured with a 0.1–200 mbar and a 1–2000 mbar manometer (Leybold). The resulting N-heterocycle/argon-mixtures were deposited onto the 12 K CsI window to a total thickness of 1 μm , controlled by standard laser interferometry. A 1 μm thick layer allowed full penetration of the UV light through the sample.

For the photolysis experiments a microwave-excited hydrogen flow lamp with a flux of 4.6×10^{14} photons $\text{cm}^{-2} \text{s}^{-1}$ was used. The UV lamp was calibrated by actinometry as described by Cottin et al. (2003) before and after each series of experiments. UV irradiation was performed in 10 second intervals to a total of 2 minutes of irradiation (5 second intervals to a total of 1 minute irradiation for s-triazine) followed by 1 minute intervals until the sample was completely destroyed. Photodestruction of the N-heterocycles was monitored with in situ Fourier-transform infrared (FT-IR) spectroscopy using an Excalibur FTS-4000 spectrometer (Bio-Rad) in the range 4000–500 cm^{-1} at 1 cm^{-1} resolution. In these experiments, the combination of an accurate gas mixing ratio, a laser-interferometry controlled sample thickness, and a calibrated lamp flux allowed for accurate and reproducible photodestruction rate measurements, which will be shown in the next section.

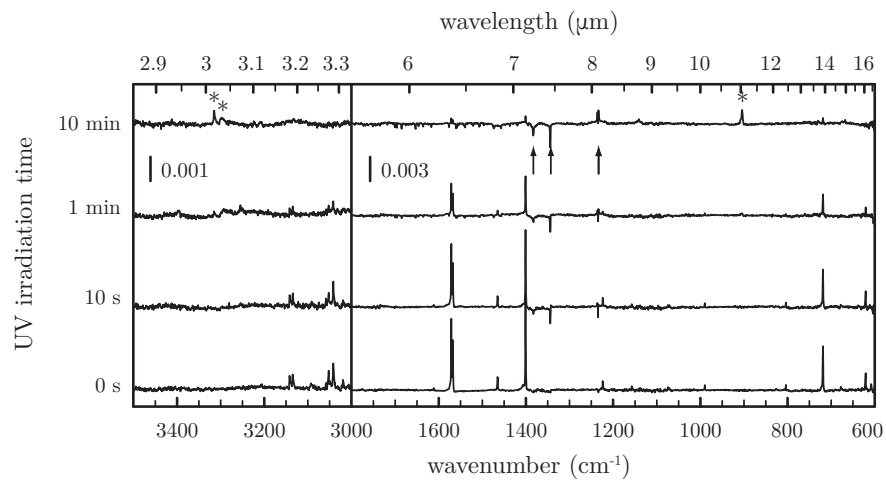
3.3 Results

The three N-heterocycles pyridine, pyrimidine and s-triazine were analysed using FT-IR spectroscopy. The resulting spectra are shown in figure 3.2. The spectra were compared with the results obtained by Destexhe et al. (1994) for pyridine and pyrimidine, and Morrison et al. (1997) for s-triazine to confirm the obtained spectra and perform band assignments, see table 3.1.

The UV photolysis of the heterocyclic molecules was measured by recording the infrared spectrum after short periods of exposure to UV radiation. In figure 3.2 the spectra after 10 sec-



(a) pyridine



(b) pyrimidine

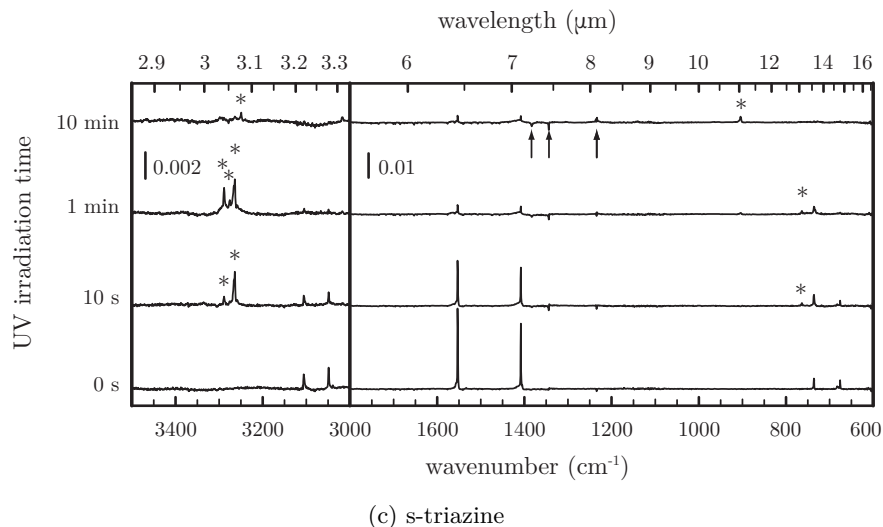


Figure 3.2

The FT-IR spectra of (a) pyridine, (b) pyrimidine and (c) s-triazine, isolated in an argon matrix at 12 K in the ranges 3500–3000 cm^{-1} (left panels) and 1800–600 cm^{-1} (right panels) with a resolution of 1 cm^{-1} . The matrix isolated molecules were irradiated with UV for 10 seconds, 1 and 10 minutes. The vertical scale bars indicate the infrared absorption in absorption units. Band assignments are presented in table 3.1. New bands appearing after photolysis are designated by asterisks (*). Photoproduct assignments are given in table 3.3. Features at 1383, 1344 and 1234 cm^{-1} , designated with arrows, are due to contamination in the system. These contaminations were accumulated outside the argon matrix and did not interfere with the photolysis reactions.

onds and 1 and 10 minutes of photolysis are shown for each N-heterocyclic molecule. The diminishing of the peaks in figure 3.2 after UV irradiation shows that all three molecules were destroyed by the UV. The rate of destruction was calculated by plotting the natural log of the normalised integrated peak areas against time and fitting the data points with a linear regression

line. Only the first 120 seconds of photolysis time (the first 35 seconds for s-triazine) were considered, because at longer photolysis times the destruction rate showed a deviation from first order behaviour.

From the slopes of the linear fits, the destruction cross-sections and half-lives were calculated, which are compiled into table 3.2. Half-

lives were calculated from the laboratory data and extrapolated to the diffuse ISM, a dense cloud and the Solar System environment at 1 AU from the Sun, using the appropriate UV fluxes: 1×10^8 photons $\text{cm}^{-2} \text{s}^{-1}$ for the diffuse ISM (Mathis et al. 1983), 1×10^3 photons $\text{cm}^{-2} \text{s}^{-1}$ for a dense cloud (Prasad & Tarafdar 1983) and 3×10^{13} photons $\text{cm}^{-2} \text{s}^{-1}$ for the solar UV flux >6 eV at 1 AU distance from the Sun. The resulting half-lives for those astronomical environments were added to table 3.2. Finally, the half-lives for the N-heterocycles were compared to benzene (Ruiterkamp et al. 2005) and plotted against the number of nitrogen atoms in the ring, resulting in figure 3.3.

The photodissociation of s-triazine in an argon matrix has been previously described by Satoshi et al. (1997). New bands appearing after 10 seconds of UV irradiation, were found at 3264.0, 2098.5, 763.7 and 732.9 cm^{-1} . These bands matched with the results of Satoshi et al. (1997) and are assigned to the C-H stretching mode, the C-N stretching mode, the in-plane bending mode and the out-of-plane bending mode of a hydrogen-bonded cyclic HCN trimer ($\text{c}-(\text{HCN})_3$), respectively. The bands of $\text{c}-(\text{HCN})_3$ reached a maximum after 30 seconds of irradiation. At longer periods of irradiation, the $\text{c}-(\text{HCN})_3$ bands disappeared again, while new bands appeared at 2295.8 and 2054.2 cm^{-1} . Using the results from Stroh & Winnewisser (1989) these two bands could be assigned to the pseudo-symmetric stretching mode and pseudo-asymmetric stretching mode of CNCN. Other bands that appeared during photolysis of s-triazine, but that could not be assigned, were

found at 3288.6, 3275.6, 3258.2 and 3249.1 cm^{-1} . All bands and their assignments are shown in table 3.3.

The change of the relative abundances of s-triazine and its photoproducts during irradiation — as measured by the integrated peak areas, relative to the highest value per peak — is shown in figure 3.4. The relative abundances in figure 3.4 were determined from the peaks at 1552 cm^{-1} for s-triazine, 3264 cm^{-1} for $\text{c}-(\text{HCN})_3$ and 2295 cm^{-1} for CNCN. From the results described above, the following s-triazine photolysis pathway is suggested: $\text{c}-\text{C}_3\text{H}_3\text{N}_3 \rightarrow \text{c}-(\text{HCN})_3 \rightarrow \text{CNCN}$, see also the inset in figure 3.4. After 30 seconds of irradiation only ~ 10 % of the initial amount of s-triazine was remaining. Assuming the suggested photolysis pathway, 90 % of the s-triazine was converted into $\text{c}-(\text{HCN})_3$ in the first 30 seconds. After this period $\text{c}-(\text{HCN})_3$ degraded with only little replenishment by further degradation of s-triazine. From the $\text{c}-(\text{HCN})_3$ peaks in the infrared spectra between 30 and 100 seconds of irradiation, the UV destruction cross-section and half-lives for $\text{c}-(\text{HCN})_3$ could be calculated in the same way as for pyridine, pyrimidine and s-triazine. The results were added to table 3.2.

In the photolysis experiments of pyrimidine only two new bands were found after 10 minutes of irradiation with UV. These bands were located at 3297.8 and 3314.7 cm^{-1} . Since s-triazine has been shown to photolyse into three HCN molecules (Satoshi et al. 1997) and benzene into acetylene molecules (Ruiterkamp et al. 2005), it can be expected that pyrimidine would degrade into two HCN molecules and one acetylene molecule. These photoproducts are likely to form a complex

Table 3.1

The wavenumbers ($\tilde{\nu}$ in cm^{-1}) of the most prominent peaks of the heterocycles with their assignments, used to monitor the UV photodestruction. ν Denotes a stretching mode, β is a bending mode and τ refers to a torsion mode.

pyridine ^a		pyrimidine ^a		s-triazine ^b	
$\tilde{\nu}$ (cm^{-1})	mode description	$\tilde{\nu}$ (cm^{-1})	mode description	$\tilde{\nu}$ (cm^{-1})	mode description
702	τ -ring	621	β -ring	680	β -ring
1031	β -ring	719	τ -ring	738	ν -ring
1441	νCC , βCH	1400	βCH , νCN	1416	νCH
1582	νCC	1571	νCN , νCC	1552	βN out-of-plane
3008	νCH	3042	νCH		

^a

[Destexhe et al. \(1994\)](#)

^b

[Morrison et al. \(1997\)](#)

Table 3.2

The UV destruction cross-sections (σ_{uv}) and half-lives in the experimental set-up in the laboratory and in the diffuse interstellar environment (DISM), dense clouds (DC) and the Solar System at 1 AU from the Sun (SoSy). Also included in this table is the cyclic hydrogen-bonded trimer of HCN, which is the first photoproduct of s-triazine. Upon continued irradiation, c-(HCN)₃ is also photolysed (figure 3.4).

	σ_{uv} (cm^2)	half-life			
		lab (s)	DISM (yr)	DC (Myr)	SoSy (min)
pyridine	1.2×10^{-17}	123	18	1.8	32
pyrimidine	2.7×10^{-17}	56	8.1	0.81	14
s-triazine	8.7×10^{-17}	17	2.5	0.25	4.4
c-(HCN) ₃	2.6×10^{-17}	57	8.3	0.83	15

Table 3.3

Wavenumbers (in cm^{-1}) of the photoproducts that appeared after UV irradiation of pyridine, pyrimidine or s-triazine. The band assignments were taken from *a* Milligan & Jacox (1973), *b* Ruiterkamp et al. (2005), *c* Satoshi et al. (1997), and *d* Stroh & Winnewisser (1989). The bands for HCN and C_2H_2 in the pyrimidine photoproducts are shifted with respect to the bands given in the references, so a complex between HCN and C_2H_2 is inferred (see text).

irradiation time	wavenumber (cm^{-1})	band assignment
pyridine		
10 min	904.1	$[\text{HAr}_2]^+{}^a$
pyrimidine		
10 min	904.1	$[\text{HAr}_2]^+{}^a$
	3297.8	C_2H_2^b (complexed with HCN)
	3314.7	HCN^b (complexed with C_2H_2)
s-triazine		
10 s	3288.6	—
	3264.0	c-(HCN) ₃ C-H stretch ^c
	2098.5	c-(HCN) ₃ C-N stretch ^c
	763.7	c-(HCN) ₃ in-plane bend ^c
	732.9	c-(HCN) ₃ out-of-plane bend ^c
1 min	3288.6	—
	3275.6	—
	3264.0	c-(HCN) ₃ C-H stretch ^c
	3258.2	—
	2098.5	c-(HCN) ₃ C-N stretch ^c
	763.7	c-(HCN) ₃ in-plane bend ^c
	732.9	c-(HCN) ₃ out-of-plane bend ^c
10 min	3249.1	—
	2295.8	CNCN pseudo-symm. stretch ^d
	2054.2	CNCN pseudo-asymm. stretch ^d
	904.1	$[\text{HAr}_2]^+{}^a$

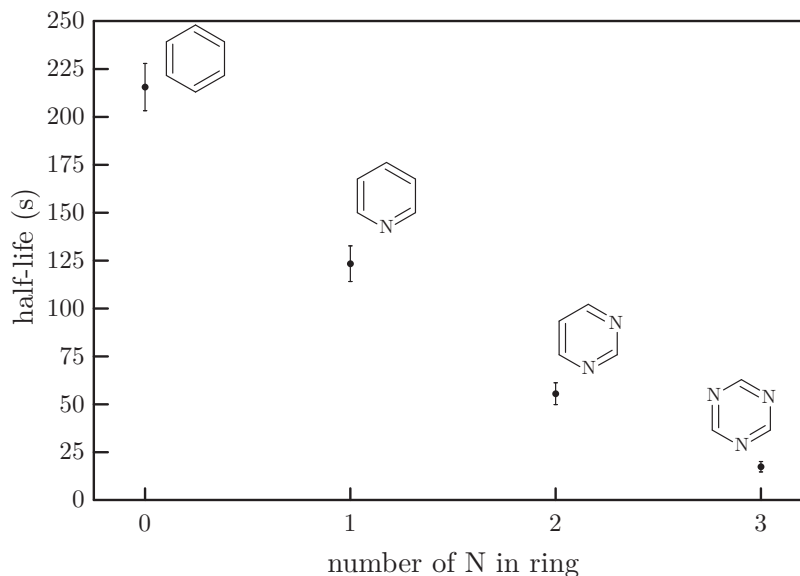


Figure 3.3

The half-lives of the three N-heterocycles plotted against the number of nitrogen atoms in the ring. Also included in this graph is the half-life of benzene, taken from [Ruiterkamp et al. \(2005\)](#). This plot shows that the photostability of small heterocyclic molecules decreases when an increasing number of nitrogen atoms is incorporated in the ring. The error bars indicate the standard deviation of the linear fit used in the calculation of the half-lives of those molecules.

in some way, since they are enclosed by an argon matrix. The peaks for the HCN monomer in an argon matrix are found at 3306 and 3202 cm^{-1} , while for a linear dimer of HCN these peaks shift to 3304 and 3210 cm^{-1} ([Satoshi et al. 1997](#)). For acetylene isolated in an argon matrix a peak is found at 3253 cm^{-1} while a peak at 3284 cm^{-1} might be assigned to a complex of multiple acetylene molecules ([Ruiterkamp et al. 2005](#)). Using

these data, the new peak at 3297.8 cm^{-1} is tentatively assigned to an acetylene mode and the peak at 3314.7 cm^{-1} to an HCN mode in a complex between acetylene and HCN.

The only new band appearing in the infrared spectra of pyridine after UV photolysis, is found at 904.1 cm^{-1} . This band is found in each experiment, including pyrimidine and s-triazine and is assigned to a proton-argon complex, $[\text{HAr}_2]^+$

(Milligan & Jacox 1973). All bands and their assignments are listed in table 3.3.

3.4 Discussion

N-heterocycles in dense interstellar and circumstellar regions

Of the 137 interstellar molecules identified to date¹, only six molecules contain 10 or more atoms, the largest being the linear carbon-chain molecule HC₁₁N. Several small ring molecules have been observed in the interstellar medium (e.g. Dickens et al. 1997, Nummelin et al. 1998). Organic ring molecules containing nitrogen atoms, c-C₂H₃N (2H-azirine, Charnley 2001) and c-C₂H₅N (aziridine, Kuan et al. 2003b, Dickens et al. 2001, Charnley 2001), have so far only been detected tentatively. Detections of large organic molecules are very difficult, due to the intrinsic weakness of their spectral lines as a result of low abundances and large partition functions. The chance of detection is further lowered for ring molecules as rings have even larger partition functions. The anticipated detectability would be even less for N-bearing heterocycles due to their expected low abundances compared to large organics containing only C, O and H. Yet another challenge for detecting these N-heterocycles could come from the contamination of the already weak target spectral lines by emission from the many interlopers from other (simpler) molecular species, present in the chemically active molecular cloud cores or circumstellar en-

velopes, the favourable sites for conducting astronomical searches of large organic molecules.

Upon irradiation of N-heterocycles with UV, these molecules are destroyed as can be seen by the extinction of their peaks in the infrared spectra in figure 3.2. The UV destruction cross-section and half-lives in table 3.2 show that this destruction proceeds rapidly under laboratory conditions. When these laboratory results are extrapolated to astrophysical conditions, pyridine, pyrimidine and s-triazine are expected to have very short lifetimes in the diffuse interstellar and Solar System environments. Only in dense interstellar clouds, where these molecules are shielded from the interstellar UV field, would pyridine and pyrimidine be able to survive the average life-span of such a cloud ($\sim 10^6$ years, Elmegreen 2000, Hartmann et al. 2001). s-Triazine, with a half-life of 250,000 years in a dense cloud environment, would have only 6 % of its initial amount remaining at the end of the cloud's lifetime. The c-(HCN)₃ complex that forms upon photolysis of s-triazine, however, has a half-life that is comparable to the half-life of pyrimidine and might survive the lifetime of a dense cloud.

Around 20 molecules have been detected in CRL618. According to models by Herpin & Cer-nicharo (2000) and Woods et al. (2002) benzene and, possibly, N-heterocycles are formed in the circumstellar envelope of CRL618. The physical structure of this circumstellar envelope is very complex. The strong UV field from the central star has produced a compact HII region that is confined by a dense circumstellar disk (Cernicharo 2004). The benzene absorption arises

¹see <http://www.astrochemistry.net>

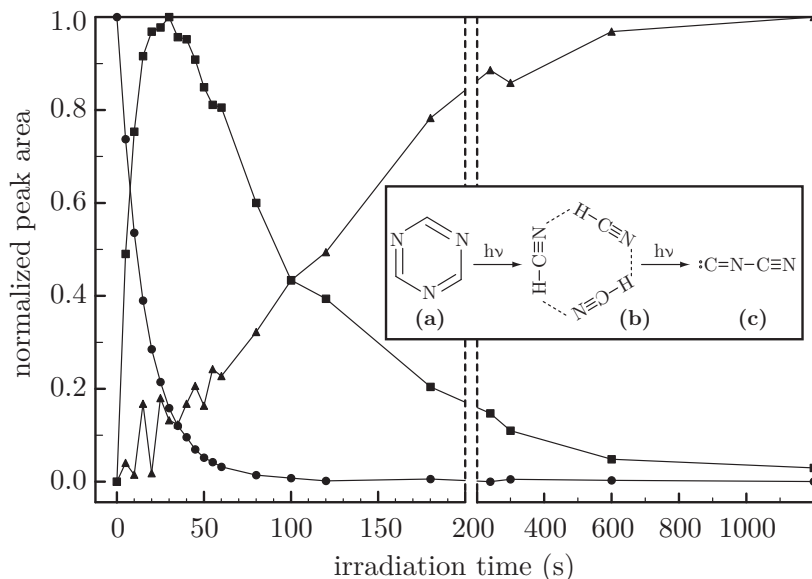


Figure 3.4

The relative column densities of s-triazine and its photoproducts during photolysis. Shown in the graph are s-triazine (graph: ●, inset: (a) measured by the 1552 cm^{-1} transition), c-(HCN)₃ (graph: ■, inset: (b) from the 3264 cm^{-1} vibration) and CNCN (graph: ▲, inset: (c) from the peak at 2295 cm^{-1}). Inset: the suggested photolysis reaction pathway for s-triazine upon irradiation with UV.

from the innermost regions of the dense circumstellar disk, where dust absorption attenuates the strong stellar UV flux. Models reported by Woods et al. (2002) indicate that when the extinction decreases to ~ 10 mag at a distance of $\sim 10^{16}$ cm from the central star, the abundances of molecules such as benzene rapidly drop, indicating destruction by interstellar UV photons. In such environments N-heterocycles would be rapidly destroyed as well.

The experiments described in this paper are performed employing the low temperature matrix isolation technique, while the results are extrapolated to the gas phase in different interstellar and circumstellar environments. Although the argon matrix provides a good spectroscopic comparison to the gas phase, there are some small differences between argon matrix and gas phase in terms of photochemistry. In the matrix, energy absorbed by the molecule can be

dissipated in the matrix, thereby lowering the chance of photodestruction. Also, after photolysis photoproducts remain in close contact with each other in the matrix, because they are enclosed by solid argon. These two factors will increase the real gas phase destruction rate (lower the gas phase half-life) and the matrix isolated destruction cross-sections presented in this paper can be regarded as lower limits (upper limits for the half-lives). [Ruiterkamp et al. \(2005\)](#) showed a factor 10 difference between gas phase and low temperature matrix isolation data for benzene.

Possible formation of N-heterocycles in space

The formation of benzene in interstellar environments could proceed via gas phase reactions or gas-grain chemistry. However, in most reaction schemes it is unclear how nitrogen atoms are incorporated into forming rings. Irradiation of icy grain analogues in the laboratory often leads to complex new molecules ([Bernstein et al. 1995, 1999, Moore & Hudson 1999](#)). The low photostability of N-heterocycles like pyridine and pyrimidine, however, may argue against the production by photochemistry on icy grains. In order to understand gas phase reactions leading to N-heterocycles, a combination of observations, theoretical models and laboratory data on the formation of benzene provides useful information.

[Woods et al. \(2002\)](#) have shown that circumstellar ion-molecule chemistry, based on the findings of [McEwan et al. \(1999\)](#), could in fact produce benzene in the abundance observed in CRL618. In this scenario, the reaction sequence

is initiated by HCO^+ reacting with C_2H_2 . A very high ionization rate (about ~ 100 times the canonical interstellar value due to cosmic ray particles) is required to produce $\text{c-C}_6\text{H}_6$ efficiently. [Woods et al. \(2002\)](#) attributed soft X-rays or shock waves as the source of enhanced ionization. In situ erosion of carbon dust to PAHs and simpler molecules, either by sputtering in shocks or by photolysis ([Wooden et al. 2004](#)), could provide a viable source of benzene to compete with its photodestruction in the diffuse ISM.

N-heterocycles are not formed as efficiently as benzene in circumstellar environments, as was discussed in section 3.1. A low formation rate combined with a low stability against UV radiation, makes the detection of N-heterocycles in circumstellar environments difficult. Nevertheless, sources with higher $\text{HCN}/\text{C}_2\text{H}_2$ ratios in the dust formation zone could have higher production rates of N-heterocycles and thus serve as better targets than CRL618 to search for these molecules. For example, the $\text{HCN}/\text{C}_2\text{H}_2$ ratio in CRL618 is observed to be about 0.16 ([Woods et al. 2002](#)), whereas the estimated $\text{HCN}/\text{C}_2\text{H}_2$ ratio around the carbon-rich AGB star IRC+10216 is about 0.6 ([Cherchneff et al. 1993](#)). This elevated $\text{HCN}/\text{C}_2\text{H}_2$ ratio, along with a much slower outflow velocity and lower UV flux, suggests that the circumstellar envelope of IRC+10216 would be a better source for finding N-heterocycles.

The high photodestruction rates make it unlikely that small N-heterocycles could have survived the conditions during Solar System formation, unless they were well protected against radiation in the disk mid-plane at all times.

N-heterocycles in meteorites

CI and CM-type carbonaceous meteorites are considered to be the most pristine samples of the early Solar System with respect to their elementary composition. However, both meteorite classes show mineralogical evidence for alteration by liquid water percolating through the parent body, evidence mainly in the form of hydrated minerals like clays, carbonates, sulphates and phyllosilicates (Endreß & Bischoff 1996). Interstellar organic matter that was trapped in the meteorite parent body during the formation of the Solar System would thus have undergone subsequent reactions in the aqueous solution to form secondary products (Zolensky & McSween 1988). Whereas early quantitative treatment of this model points to temperature estimates of less than 20 °C for CM and 100–150 °C for CI carbonaceous chondrites (Clayton & Mayeda 1984), refinements of the models reduced the temperature for the CIs to ~50 °C (Leshin et al. 1997). A pH in the range of 6 to 8 has been suggested for the aqueous phase of CM chondrites (DuFresne & Anders 1962).

It is generally accepted that the amino acids found in carbonaceous meteorites, are produced during the phase of aqueous alteration in the parent body. These compounds can be synthesized from interstellar precursor molecules such as HCN, ammonia and carbonyl compounds, including formaldehyde, acetaldehyde and acetone. This formation route, the so-called Strecker-cyanohydrin reaction, can take place inside the meteorite parent body during aqueous alteration (Cronin & Chang 1993). These conditions could also be favourable for the formation of purines

and pyrimidines.

The first synthetic step towards the abiotic synthesis of purines could be HCN polymerisation, which would lead to the formation of the HCN-tetramer diaminomaleonitrile (DAMN) as an intermediate. This compound, which is obtained in yields of 0.3 to 0.5 % in aqueous 0.1–1.0 M HCN solutions, is also a possible intermediate for the formation of certain amino acids (Feris & Hagan 1984). The formation of DAMN requires basic pH values (9.2 in the laboratory experiment), while the liquid inside the meteorite parent bodies is thought to be buffered to pH 6 (slightly acidic) by the mineral composition present in the meteorite (DuFresne & Anders 1962).

Several possible pathways exist for the reaction of DAMN with other compounds such as ammonia and/or formamide to lead to the formation of adenine, the simplest purine. The reaction of DAMN with urea leads to the formation of guanine and xanthine in yields of 5–10 %. Another prebiotic pathway for the formation of the adenine derivative 8-hydroxymethyladenine (HMA) as well as traces of adenine from HCN and formaldehyde, was experimentally demonstrated by Schwartz & Bakker (1989). Finally, it has been demonstrated recently that guanine can be produced from ammonium cyanide polymerizations (Levy et al. 1999).

The abiotic synthesis of pyrimidines is more difficult to achieve. The first proposal for the formation of a pyrimidine from a cyano-compound was the synthesis of cytosine from cyanoacetylene and cyanate, both possibly prebiotic compounds, followed by hydrolysis, leading to uracil

(Ferris et al. 1968). Starting from DAMN, a variety of pathways for the formation of pyrimidine derivatives, for example the reaction of DAMN with guanidine that yields 5-hydroxyuracil, were proposed by Ferris et al. (1978). Only uracil was detected in carbonaceous chondrites (Stoks & Schwartz 1979), a fact that may reflect the apparent difficulty in the abiotic synthesis of these compounds.

In summary, carbonaceous chondrites appear to contain several classes of N-heterocyclic compounds, including purines, pyrimidines, quinolines/isoquinolines and pyridines. Stable isotope data of these compounds, which would be very helpful in determining their extraterrestrial origin, have yet to be reported.

3.5 Conclusion

Pyrimidine is a precursor of the biologically important nucleobases. Together with two other nitrogen-containing heterocyclic molecules pyridine and s-triazine, the UV destruction rate was determined using low temperature matrix isolation in solid argon. We found that these molecules were rapidly destroyed when photolysed with UV radiation and that the stability decreased with an increasing number of nitrogen atoms in the ring. When scaled to the UV fluxes of astronomically relevant environments, these molecules would be destroyed fast in the UV dominated regions of circumstellar envelopes and diffuse interstellar clouds. Only in dense clouds could pyridine and pyrimidine, but not s-triazine, survive the average lifetime of the cloud. From these results, combined with the

weak spectral lines and low abundances of these N-heterocycles, we expect a low probability for the detection for these molecules. The most promising search site for N-heterocycles would be targets with a high HCN/C₂H₂ ratio, such as the AGB star IRC+10216. However, N-heterocyclic compounds have been detected in carbonaceous meteorites. The low probability for these compounds to survive in the gas phase in the interstellar medium and during planetary system formation, as strongly indicated by the results of this study, suggest that these N-heterocycles were probably synthesised on the meteorite parent bodies.

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Four

The astrobiology of nucleobases

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Abstract

Nucleobases are nitrogen heterocycles (N-heterocycles) which are essential components of the genetic material in all living organisms. Extraterrestrial nucleobases have been found in several carbonaceous chondrites, but only in traces. No astronomical data on these complex molecules are currently available. A large fraction of the cosmic carbon is known to be incorporated into aromatic material and, given the relatively high abundance of cosmic nitrogen, the presence of N-heterocycles can be expected. We present infrared spectroscopic laboratory data of adenine and uracil under simulated space conditions. At the same time we tested the stability of these nucleobases against ultraviolet (UV) irradiation at 12 K. Our experimental results indicate that gas phase adenine and uracil will be destroyed within hours in the Earth's vicinity. In dense interstellar clouds exposed to UV radiation, only adenine could be expected to survive for a few million years. We discuss possible formation routes to purines and pyrimidines in circumstellar environments and in meteorite parent bodies.

4.1 Introduction

Many complex organic molecules have been detected in the interstellar medium (ISM) and in solar system objects in the solid state or dispersed in the gas phase (see [Ehrenfreund & Charnley 2000](#), for a review). The dominant fraction of cosmic carbon is incorporated in aromatic material, likely in gaseous polycyclic aromatic hydrocarbons (PAHs) and in the solid aromatic networks which comprise carbonaceous dust. The ubiquitous signatures of aromatic material visualized by infrared emission bands between 3–15 μm also provide evidence for the presence of five-membered ring structures and attached side groups ([Tielens et al. 1999](#)). Nucleobases such as purines and pyrimidines are small N-containing aromatic ring structures which play a major role in terrestrial biochemistry. They are central components of DNA and RNA, molecules that are used in the storage, transcrip-

tion and translation of genetic information. At present, extraterrestrial N-heterocycles have only been detected in carbonaceous meteorites ([Stoks & Schwartz 1979, 1981](#)). In this Letter we report experimental results on the spectroscopy and photostability of adenine and uracil. We also evaluate possible nucleobase formation mechanisms in a variety of astronomical environments.

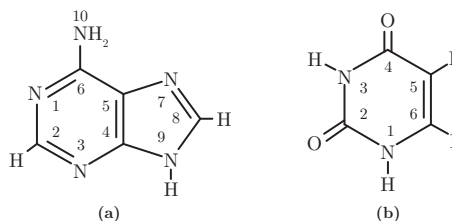


Figure 4.1

The structural formulae of (a) adenine ($\text{C}_5\text{H}_5\text{N}_5$) and (b) uracil ($\text{C}_4\text{H}_4\text{N}_2\text{O}_2$).

Where could nucleobases be formed in space?

There are several tentative formation routes of nucleobases in interstellar, circumstellar and solar system environments. Currently there are no astronomical data available to identify nucleobases in interstellar or circumstellar environments. Theoretical scenarios for the interstellar formation of prebiotic compounds in the ISM have most recently been discussed by [Charnley et al. \(2001\)](#), [Chakrabarti & Chakrabarti \(2000\)](#), [Hollis & Churchwell \(2001\)](#), [Sorrell \(2001\)](#). [Chakrabarti & Chakrabarti \(2000\)](#) suggested a gas phase formation of adenine in molecular clouds. However, this route to the formation of nucleobases in dense molecular clouds and star-forming cores can be ruled out based on the calculations of [Smith et al. \(2001\)](#). The computed rate coefficient for HCN dimerization, the first step on the proposed formation route of [Chakrabarti & Chakrabarti \(2000\)](#), is $\sim 10^{-10} \exp(-36,000/T) \text{ cm}^3 \text{ s}^{-1}$ and implies that adenine formation will not occur in such regions, where temperatures are in the range of $T \sim 10\text{--}300 \text{ K}$. Also, adenine could be formed in the inner circumstellar envelopes of C-stars ($T \sim 1000\text{--}1500 \text{ K}$), where formation of aromatic compounds is known to occur ([Frenklach & Feigelson 1989](#)). In this case, N-heterocycles may form as by-products of the acetylene polymerization which leads to large PAH molecules.¹

Intermediate HCN additions could lead to direct incorporation of nitrogen atoms into growing aromatic rings ([Ricca et al. 2001](#)). For exam-

ple, in the initial stage of ring closure to form a phenyl radical ($\text{C}_6\text{H}_5\cdot$), an HCN addition, instead of an acetylene addition, will lead to the formation of pyridine. Similarly, the replacement of an acetylene by an HCN during the subsequent formation of naphthalene would lead to isoquinoline. Molecules formed in this scenario will preferentially contain one nitrogen atom per aromatic ring rather than several nitrogen atoms in one ring as in adenine, making this mechanism an inefficient source of adenine. The presence of two oxygen side-groups in uracil rules out its formation in C-star atmospheres.

Irradiation of PAH molecules embedded in H_2O ice matrices leads to the formation of various carbonyl compounds such as quinones and ethers ([Bernstein et al. 1999](#)). However, these experiments showed no inclusion of oxygen into the aromatic ring structure of the PAHs; side-group additions are preferred ([Bernstein et al. 2003](#)). The situation is unknown for irradiation of PAHs in nitrogen-rich ices, but a highly efficient mechanism would have to operate to produce such a nitrogen rich molecule as adenine. Irradiation of interstellar pyrimidine ($\text{c-C}_4\text{H}_4\text{N}_2$) in water ice could possibly produce the necessary side-group additions for uracil. However, existing upper limits on the abundance of interstellar pyrimidine ([Kuan et al. 2003](#)) tend to greatly constrain the amount of pyrimidine available for this synthesis. For both adenine and uracil, the experimental data reported in this Letter limit the possibility that these molecules can survive in environments where a significant UV flux is present.

¹Note that HCN dimerization in these environments is also ruled out by the calculations of [Smith et al. \(2001\)](#).

As traces of nucleobases have been identified in carbonaceous meteorites, the formation of nucleobases within the solar system appears to be more promising. No isotopic measurements have been made for any N-heterocyclic compound found in meteorites that would provide definitive evidence for their extraterrestrial origin. However, based on the very low contamination levels of amino acids in these meteorites, a low terrestrial contamination for the nucleobases can be inferred. Indigenous purines and pyrimidines have been detected in several carbonaceous chondrites. The pyrimidine uracil and the purines adenine, guanine, xanthine and hypoxanthine (Stoks & Schwartz 1979, 1981) were detected in the CM carbonaceous chondrites Murchison and Murray, as well as in the CI meteorite Orgueil, in total concentrations of about 1.3 ppm. Upper limits exist (detection limit 0.01 ppm) for the concentrations of thymine and cytosine, as well as other heterocyclic compounds, in the Murchison meteorite (van der Velden & Schwartz 1977).

The synthetic pathways for the formation of nucleobases in a meteoritic parent body might be similar to those suggested for their prebiotic formation on the early Earth. In this scenario stepwise oligomerization of HCN leads to the formation of the tetramer diaminomaleonitrile (DAMN). Subsequent steps lead to the formation of 4-aminoimidazole-5-carbonitrile (AICN) (Ferris & Hagan 1984). This intermediate can either react with HCN to form adenine and hypoxanthine or with urea to form guanine and xanthine. All these reactants are potential prebiotic compounds, however only HCN has been identified

in the ISM and in comets.

4.2 Experimental

Matrix isolation spectroscopy and subsequent UV destruction of adenine and uracil was carried out using techniques described elsewhere (Ehrenfreund et al. 2001). For the matrix material 99.999 % pure argon (Praxair) was used. Adenine (purity of 99 %) was purchased from Merck and uracil (purity of 98 %) was purchased from Sigma. The nucleobase samples were sublimed from a furnace at 100 °C onto a 12 K CsI window. Simultaneously, the matrix gas was deposited onto the window from a separate inlet port at a rate of 3.0×10^{19} molecules cm^{-2} hour^{-1} or $11.3 \mu\text{m hour}^{-1}$. The column densities of adenine and uracil were estimated from the most prominent bands. The mode descriptions and associated band strengths were taken from Nowak et al. (1996) for adenine and from Leś et al. (1992) for uracil. The nucleobase to argon ratio in the resulting matrix was $\sim 1:1500$.

UV irradiation was performed using a microwave excited hydrogen flow lamp with a flux ϕ of 5×10^{14} photons cm^{-2} s^{-1} , calibrated according to the method described by Gerakines et al. (2000). Photodestruction of the nucleobase samples was monitored by in situ FT-IR spectroscopy using an Excalibur FTS-4000 (Bio-Rad) at 1 cm^{-1} resolution. Column densities were calculated from integrated peaks and plotted against time. These data were fitted with the function $N_0 e^{-kt}$, where N_0 is the initial (before photolysis) column density in molecules cm^{-2} , and t the time of irradiation in seconds, yielding

the destruction rate constant k in s^{-1} . Furthermore $k = \sigma_{\text{uv}} \phi$, where σ_{uv} is the UV destruction cross section in $\text{cm}^2 \text{ molecule}^{-1}$. Finally, half-lives — defined as the amount of time required to destroy 50 % of the starting material — were calculated for different environments, using the known UV fluxes for laboratory conditions, the diffuse interstellar medium (DISM, $1 \times 10^8 \text{ photons cm}^{-2} \text{ s}^{-1}$, [Mathis et al. 1983](#)), dense clouds (DC, $1 \times 10^3 \text{ photons cm}^{-2} \text{ s}^{-1}$, [Prasad & Tarafdar 1983](#)), and the Sun at 1 AU ($3 \times 10^{13} \text{ photons cm}^{-2} \text{ s}^{-1}$), see table [4.1](#).

4.3 Results and Discussion

The UV destruction of adenine and uracil in solid Ar at 12 K was measured by monitoring the disappearance of spectral features associated with specific molecular functional groups. For adenine the relevant bands are found at 3557 cm^{-1} (NH_2), 3498 cm^{-1} ($\text{N}(9)\text{H}$), 3441 cm^{-1} (NH_2), between $1651\text{--}1599 \text{ cm}^{-1}$ ($\text{C}=\text{C}$, $\text{C}=\text{N}$), 1474 cm^{-1} ($\text{C}=\text{N}$, $\text{C}-\text{H}$), 1290 cm^{-1} ($\text{C}=\text{N}$) and 803 cm^{-1} (ring-mode). Figure [4.2](#) shows the degradation of adenine upon photolysis in defined time steps of 1, 10 and 30 minutes, after which nearly all bands have disappeared. Photo-products identified at 904 cm^{-1} and 1092 cm^{-1} have been tentatively assigned to HAr_n^+ ([Milligan & Jacox 1973](#)) and the ν_3 absorption of HO_2 ([Jacox & Milligan 1972](#)), respectively; these may result from residual water contamination in the setup. No likely candidate could be identified for a third feature which appeared at 1141 cm^{-1} .

Figure [4.3](#) shows the destruction of the pyrimidine compound uracil. The functional

group specific signals of uracil are at 3485 cm^{-1} ($\text{N}(1)\text{H}$), 3435 cm^{-1} ($\text{N}(3)\text{H}$), between $1792\text{--}1681 \text{ cm}^{-1}$ ($\text{C}=\text{O}$), 1399 cm^{-1} ($\text{N}-\text{H}$, $\text{C}-\text{N}$), 1185 cm^{-1} ($\text{C}-\text{H}$, $\text{N}-\text{H}$), 804 cm^{-1} ($\text{C}(4)\text{O}$, $\text{C}(5)\text{H}$), and 757 cm^{-1} ($\text{C}(2)\text{O}$). After 20 minutes of irradiation all uracil specific peaks disappeared while new peaks emerged at 904 cm^{-1} , 1092 cm^{-1} , 1141 cm^{-1} , 2143 cm^{-1} , 2263 cm^{-1} , and 2345 cm^{-1} . The first three peaks have the same tentative assignment as for the adenine photolysis. The peaks at 2143 cm^{-1} and 2345 cm^{-1} are the well described stretching modes of CO and CO_2 , respectively. The band at 2263 cm^{-1} is tentatively ascribed to the ν_2 absorption of HNC (Bondybey et al. 1982). Isolated water bands around 3700 cm^{-1} are marked in figures [4.2](#) and [4.3](#). The water content has been estimated from those bands using the cross sections given by Redington & Milligan (1962) and has been assessed to be negligible, namely $4.0 \times 10^{14} \text{ molecules cm}^{-2}$. The UV radiation has been performed in a careful experimental procedure ensuring that the sample thickness of the Ar layer does not exceed $1 \mu\text{m}$ allowing full penetration of all UV photons throughout the ice layer. From the structural formula of uracil it seems apparent that by breaking the bonds between C(2) and N(3), between C(4) and C(5), and between C(6) and N(1) (see figure [4.1](#)), photolysis would yield 2 HNC molecules per uracil, which are identified by the 2263 cm^{-1} band in the spectrum. The remainder could then form acetylene (C_2H_2). However, no acetylene was found in the matrix. For adenine no infrared active photolysis products were found. The degradation routes for both adenine and uracil will be investigated in future research.

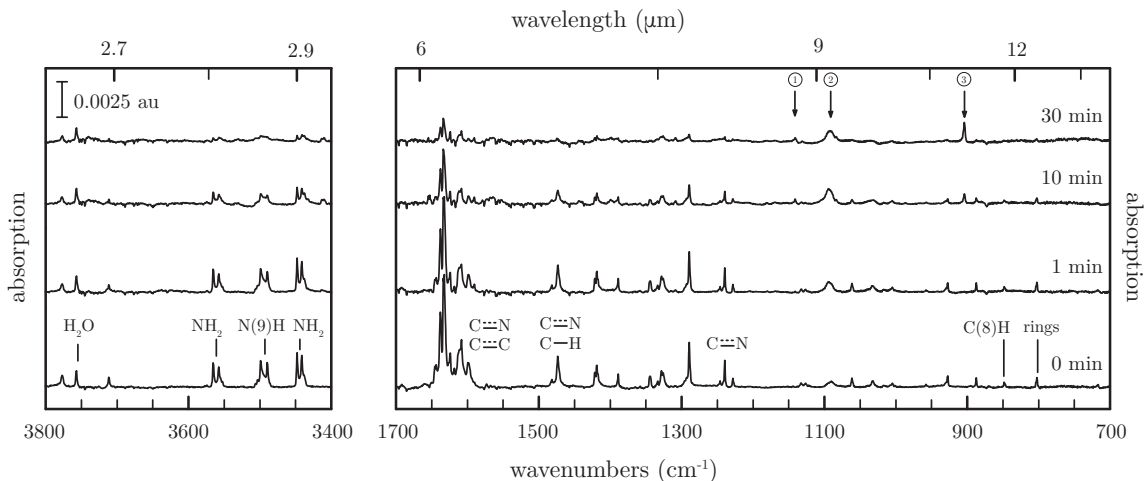


Figure 4.2

Infrared spectra of adenine isolated in argon (1:1500) at 12 K in the ranges 3800–3400 cm^{-1} and 1700–700 cm^{-1} (au=absorption units). The major infrared transitions are labelled. The spectra were recorded after deposition (0 min) and 1, 10 and 30 minutes of exposure to UV radiation. The peaks that emerge after UV photolysis are depicted with arrows, labelled ① at 1141 cm^{-1} , ② at 1093 cm^{-1} , and ③ at 904 cm^{-1} .

From the disappearance of the major features in the adenine and uracil spectra over time, half-lives were calculated for several different environments. The results are listed in table 1. Also incorporated in table 1 is the half-life for the amino acid glycine. The photostability of glycine measured in this experiment is lower than previously measured by Ehrenfreund et al. (2001). The current values are believed to be more accurate due to the controlled thin sample thickness in our experiments. The stability of uracil was found to be rather low, only slightly higher than glycine. Adenine, however,

showed a 5 times higher stability against UV irradiation than glycine. In contrast to the efficient photodestruction nucleobases undergo, larger N-substituted PAHs exhibit much greater photostability and can become photoionized (Mattioda et al. 2003). This means that any ionization products in our experiments cannot be effectively monitored, and will only make a minor contribution to the loss of IR absorption.

The experimental data for argon matrices provide upper limits for the survival of nucleobases in specified regions of space. From table 1 it is clear that, even if nucleobases are

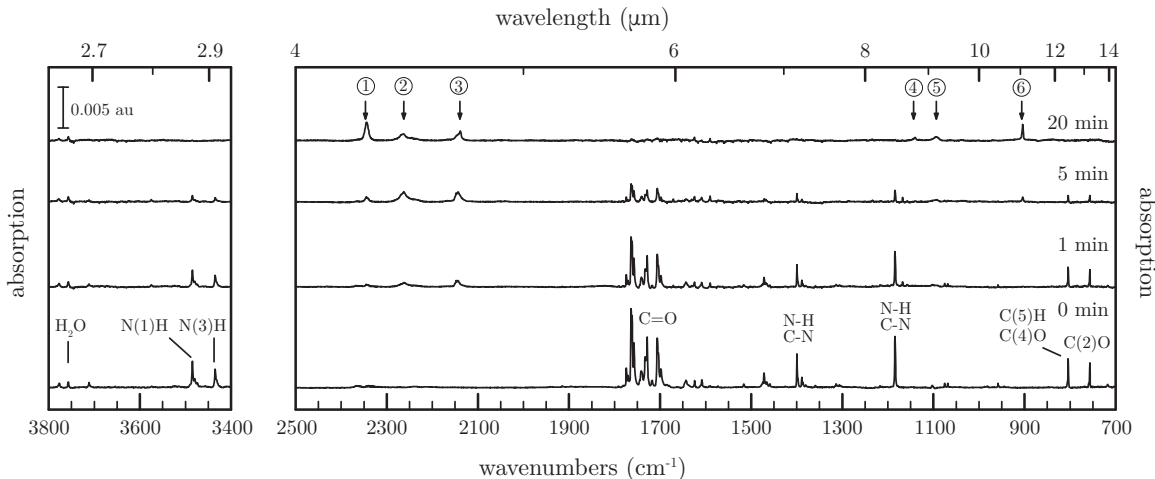


Figure 4.3

Infrared spectra of uracil isolated in argon (1:1500) at 12 K in the ranges 3800–3400 cm^{−1} and 2500–700 cm^{−1} (au=absorption units). The major infrared transitions are labelled. The spectra were recorded after deposition (0 min) and 1, 5 and 20 minutes of exposure to UV radiation. The peaks that emerge after UV photolysis are depicted with arrows, labelled ① at 2345 cm^{−1}, ② at 2263 cm^{−1}, ③ at 2143 cm^{−1}, ④ at 1141 cm^{−1}, ⑤ at 1093 cm^{−1}, and ⑥ at 904 cm^{−1}.

formed around evolved stars, they could not survive in the diffuse interstellar medium for more than several hundred years. Near the Earth, at 1 AU from the Sun, adenine and uracil would be destroyed in a matter of hours. The average lifetime of shielded environments like molecular clouds is uncertain. Short cloud lifetimes of ~1–3 Myr (e.g. Elmegreen 2000, Hartmann et al. 2001) favour the survival of all three molecules. For longer lifetimes of ~10 Myr, only adenine could be expected to survive over a significant fraction of a cloud’s life, e.g. over the age of the Taurus

cloud complex (~6 Myr, Cohen & Kuhi 1979).

4.4 Conclusions

Taking into account our current understanding of the relevant chemistry it is doubtful that nucleobase formation can proceed efficiently in circumstellar and interstellar environments. Although the photostability of adenine is a factor of five larger than for uracil and glycine, it is still sufficiently low to limit its life-time in such

Table 4.1

Destruction cross sections and half-lives for the nucleobases and glycine. This table lists the destruction cross section and half-lives for the nucleobases and glycine in different environments. The destruction cross section was calculated by monitoring the disappearance of infrared transitions in figures 4.2 and 4.3. The half-lives were subsequently calculated using the specific UV flux for the given environment (see text).

compound	σ_{uv} (cm ² per molecule)	half-life			
		Lab (s)	DISM (yr)	DC (Myr)	1 AU (s)
adenine	2.7×10^{-18}	518	82.7	8.27	8654
uracil	1.1×10^{-17}	127	20.3	2.03	2120
glycine	1.2×10^{-17}	115	18.4	1.84	1925

environments. Their instability against UV photons also implies that photochemical production on the surface of interstellar grains will be inefficient.

It therefore seems most likely that nucleobases can only be formed in the Solar System, where they could be readily synthesized from common extraterrestrial starting materials such as hydrogen cyanide and cyanoacetylene (Ferris & Hagan 1984). Ideal environments in which to form nucleobases such as adenine and uracil are the parent bodies of meteorites. Comets may also be a formation site if HCN polymerization is invoked. The formed nucleobase compounds could then be protected from UV photons in cometary subsurface layers. Their survival time in the solar system is extremely limited when unprotected against UV radiation, only several hours in the vicinity of the Earth. It can be concluded that

the precursors of terrestrial biomolecules, such as amino acids and nucleobases, are not very resistant against UV radiation and that these compounds require protective conditions for them to be formed, transported and delivered to the early planets.

Acknowledgements

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Five

**Mars regolith analogues: chemical properties and their effects
on amino acids under simulated martian conditions**

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Abstract

Recent missions to Mars have provided strong evidence for the presence of liquid water in the past on the martian surface. The presence of liquid water for a geologically relevant period of time suggests the possibility that life may have emerged on early Mars, given an appropriate energy source (e.g. the Sun) and a source of simple organic compounds. If life arose on Mars, evidence in the form of complex organic compounds may still be present. A source for organic compounds on Mars should be the extraplanetary delivery by comets and carbonaceous meteorites, but evidence for their presence was not found by the Viking landers. One hypothesis to explain the absence is that there may be degradation processes occurring in or on the martian regolith that destroy organic compounds, or alter them in such a way that they are not detected.

In this paper we describe the investigations into the chemical properties of Mars soil analogues and their influence on the stability of amino acids. We collected soil samples from the Atacama desert near the Arequipa region in Peru and near the Yungay region in Chile. A precipitated sediment from the Salten Skov region in Denmark and a sample of the Orgueil meteorite, were also included. We measured the pH, redox potential, ion concentrations, carbonaceous compounds, and amino acid abundances of various samples. The results show large differences for the measured properties, even for samples taken only several metres apart. The Arequipa, Salten Skov, and Orgueil samples were exposed to a simulated Mars environment to test the stability of amino acids in these Mars soil analogues. The results show moderate to strong levels of amino acid destruction for the Arequipa and Orgueil samples, but not for the Salten Skov sample. The difference in amino acid stability is attributed to differences in the mineralogical composition among the samples. Our results are interpreted in the context of future missions to Mars that will be searching for organic molecules embedded in mineral matrices.

5.1 Introduction

Data returned from recent Mars mission indicate that water was abundant on the surface of Mars in the past (e.g. [Heldmann et al. 2007](#)). The appearance of gullies on the surface of Mars is attributed to ground water seepage and surface runoff ([Malin & Edgett 2000](#)). Hematite and jarosite, which on Earth are typically formed in an aqueous environment or by aqueous alteration, have been found by the Mars Exploration

Rovers ([Squyres et al. 2004](#)). The presence of large deposits of phyllosilicates suggests weathering of the basaltic crust by liquid water ([Chevrier & Mathé 2007](#)). Cementation and bleaching along joints in layered deposits have been discovered in observations from the Mars Reconnaissance Orbiter ([Okubo & McEwen 2007](#)), indicating fluid alterations in the geological past by subsurface flows.

The presence of liquid water for a geologically relevant period of time suggests the possi-

bility that life could have emerged on Mars. If life arose in that period, evidence in the form of organic compounds might still be present on Mars today. However, the 1976 Viking landers, the only spacecraft to date to have searched specifically for organic molecules on Mars, did not detect any organic compounds above a detection limit of a few parts per billion (ppb) within 30 cm below the surface (Biemann & Lavoie 1979). The failure to detect organic compounds is generally attributed to the intense UV radiation and highly oxidizing and acidic nature of the martian surface environment that would destroy most organic compounds in a short period of time (Benner et al. 2000, Squyres et al. 2004).

Several models for the degradation of organics in the martian soil have been suggested; most models require UV radiation or oxidants, or a combination of the two. The atmosphere of Mars allows solar light down to ~ 190 nm to reach the surface of the planet, see Patel et al. (2002) for a model of the martian lighting conditions. The direct impact of UV light on the surface of Mars can degrade small organic compounds directly (Stoker & Bullock 1997, ten Kate et al. 2005). However, the martian soil blocks UV photons within the first few millimetres and any organics that might be present in the soil below are shielded from the UV radiation.

UV light may also photolyse water molecules in the atmosphere and create reactive species like H, OH, and HO₂ radicals and H₂O₂. This process is especially effective in the upper atmosphere, where more energetic UV radiation (below 190 nm) is present. The reactive species can diffuse down into the regolith where they

can oxidize organic molecules. Finally, oxidizing species may also be created directly in the soil, by Fenton-type reactions (Liang et al. 2006).

Energetic solar protons and galactic cosmic ray particles can penetrate to a much greater depth than UV radiation. Dartnell et al. (2007) modelled that *E. coli*, *B. subtilis*, and *D. radiodurans* would be killed by such radiation at 2 m depth in 30,000, 250,000, and 450,000 years, respectively. Organic molecules are susceptible to degradation by MeV protons (Ruiterkamp et al. 2005), although larger organic molecules like polycyclic aromatic hydrocarbons (PAHs) are altered rather than destroyed (Bernstein et al. 2003).

The upcoming Mars Science Laboratory and ExoMars missions will be searching for organics in the martian regolith. In order to direct those and future missions to localities on Mars where organic compounds are likely to be found, a good understanding of the interactions between organic compounds and the regolith is necessary. The goal of this paper is to characterize soil samples, which are used as Mars regolith analogues. We have performed experiments to measure the chemical properties of the soil samples and to test the stability of amino acids in those samples under simulated martian conditions. We conclude that the interaction between organic molecules and the surrounding mineral matrix is the key to understand the survival of organics on Mars.

5.2 Martian soil analogues

Yungay

Soil samples of the Atacama desert were obtained from the Yungay region in Chile at a site named *Flat Top Hill* (S25°29'50.9" W69°50'22.5"). A second sample was taken from an excavation (*Soil Pit*, S24°06'06.0" W70°01'05.6"), at a depth of 10 cm. The layered structure of the Soil Pit was previously measured by [Ewing et al. \(2006\)](#), who determined a mineral composition of gypsum, quartz, and anorthite. Reports on the number of culturable microorganisms in the Atacama desert soil vary from near sterility (<10 CFU g⁻¹, [Cameron 1969b,a](#), [McKay et al. 2003](#)) to 10^7 CFU g⁻¹ ([Cameron & Blank 1965, 1966](#), [Glavin et al. 2004](#)), and from $\sim 10^2$ to 10^5 CFU g⁻¹ for subsurface samples ([Maier et al. 2004](#)). More recently, [Lester et al. \(2007\)](#) found a biomass between 8.5×10^6 and 6.0×10^7 cells g⁻¹ based on phospholipid fatty acid (PLFA) measurements, and between 6.3×10^2 and 5.2×10^3 CFU g⁻¹ of culturable biomass. They also found a very low total organic content (TOC), between 560 and 765 µg g⁻¹.

Arequipa

The Arequipa region in Peru is a northern extension of the Atacama desert. From the Arequipa region, several soil samples were collected on the top of a small slope (S16°44'34.7" W72°02'37.9", altitude 1165 m), where the top soil was taken (*Top of Slope*). Another top-layer sample was taken at the foot of the same slope, about 10 m downhill (designated *Foot of Slope*). A sec-

ond site was visited 6 km north-east of the first site (S16°41'41.2" W72°01'01.8", altitude 1165 m). The samples obtained here are referred to as *Second Site*. The soil names and locations are summarized in table 5.1.

Salten Skov

The Salten Skov soil is a finely powdered, dark red precipitated sediment, obtained from the epinamous region in Jutland, Denmark. Originally sampled from two sediment intervals (0–20 cm and 21–65 cm, [Nørnberg et al. 2004](#)), we used only the top-layer sample that contains a high silt and clay fraction (~ 35 % of the grain size is <63 µm). It is composed mainly of goethite with 5.5 wt% maghemite and 12.8 wt% hematite ([Nørnberg et al. 2004](#)). Fresh Salten Skov samples contain 1.5×10^6 colony forming units (CFU) g⁻¹ under aerobic conditions ([Hansen et al. 2005](#)).

Orgueil

Although not available in bulk for use as a soil analogue, a sample of the carbonaceous meteorite Orgueil was included in the Mars simulation experiment. Orgueil is known to contain high abundances of hydrous minerals (e.g. [Miyamoto & Zolensky 1994](#)) and organic compounds (e.g. [Ehrenfreund et al. 2001](#), [Pizzarello & Huang 2002](#), [Martins et al. 2006](#)). This CI1 meteorite shows a simple amino acid distribution, with β-alanine and glycine as the most abundant amino acids ([Ehrenfreund et al. 2001](#)). It also has a simple free carboxylic acid content, containing exclusively aromatic acids ([Martins et al. 2006](#)). [Flynn \(1996\)](#) calculated that fine-grained mete-

Table 5.1

the martian soil analogues and the locations from where they were obtained. The short names in the first column will be used throughout this paper to refer to the samples.

short name	location	coordinates
Top of Slope	Arequipa, Peru	S16°44'34.7" W72°02'37.9"
Foot of Slope	Arequipa, Peru	S16°44'34.5" W72°02'38.0"
Second Site	Arequipa, Peru	S16°41'41.2" W72°01'01.8"
Flat Top Hill	Yungay, Chile	S25°29'50.9" W69°50'22.5"
Soil Pit	Yungay, Chile	S24°06'06.0" W70°01'05.6"
Salten Skov	Salten Skov, Denmark	—

oritic material in the range of 0.1–27 μm may supply Mars with up to 2.4×10^5 kg of carbon yr^{-1} . Larger meteorites may arrive in tact on Mars’ surface only in a small range of masses (10–50 g [Bland & Smith 2000](#)). Once these meteorites have fallen on Mars, they suffer physical and chemical alterations (i.e. weathering) exposing their organic content to the martian environment, although weathering might proceed slowly for the larger meteorites ([Bland & Smith 2000](#)). In order to determine the stability of organic compounds on exposed meteoritic surfaces under simulated Mars conditions, samples of the Orgueil meteorite were included.

5.3 Experimental

Soil properties

Oxidation-reduction potential (E_{H}) and pH measurements were taken using an Orion 635 MMSTM

Meter with an Orion 61-79 pHutureTM combination QuatrodeTM (pH/ORP/temperature) electrode with Orion pHuture Sure-Flow Reference Probe Filling Solution (610011). For pH calibrations, premixed Thermo-Orion buffer solutions were used (pH 4, 7 and 10) and for ORP calibration, Thermo-Orion ORP standard was used to calibrate to the standard hydrogen electrode. The probe uses an integral temperature sensor to automatically compensate measurements for temperature. Measurements were made by adding soil sample to high-purity (18 M Ω) Milli-Q water in a 1:2 (wt:wt) soil-to-water ratio. In our experimental method, we begin measuring the E_{H} -pH of the soil as it is introduced to water and continue taking measurements as a function of time until equilibrium is approached. Ultra-high purity nitrogen gas was used to purge the soil-solutions. The electrode performance and measured E_{H} values were validated using a series of standard quinhydrone solutions. The sol-

uble ion content of the soils was determined at the NASA Ames Analytical Laboratory by aqueous solvent extraction using a 1:2 (wt:wt) soil-to-water ratio and analysis by ion chromatography.

Laser desorption–mass spectroscopy

Laser desorption (linear) time-of-flight (TOF) mass spectrometry (LD–MS) was used for the detection of organic content in the desert soil samples. A μL of concentrated suspension of the soil in doubly distilled water was placed on a stainless steel disk and transferred by a rapid sample change port into the high vacuum chamber ($\sim 2 \times 10^{-7}$ – 2×10^{-8} mbar) of a Kratos vacuum MALDI–MS. Neutral and ionized particles were desorbed using a 337 nm (UV) nitrogen laser at low laser densities ($\sim 10^6 \text{ W cm}^{-2}$). Mass spectra of positive ions emitted directly in the desorption process, were collected at low laser fluences. Blanks were run between each sample analysis. A blank consisted of a spot on the sample disc that has had no prior sample extract introduced, or a spot of pure doubly distilled water. To ensure statistical accuracy and reproducibility, the spectra were averaged over several 100 laser shots. Previous studies of meteorite samples under identical laser conditions have demonstrated statistical accuracy and reproducibility to within $\sim 3\%$ of the measured standard, averaged over 100 shots.

Mars simulation experiment

Three Mars soil analogues were selected for the simulation experiments: a desert soil (the Top of Slope sample from the Arequipa region), a

sediment (Salten Skov), and a meteoritic sample (Orgueil). Serpentine was included in the simulation experiment as a control blank. The serpentine sample was ground to a powder in a glove box under a flow of ultra high purity argon, using a ceramic mortar and pestle. The serpentine was then heated to 500°C for 3h.

The stability of amino acids in the selected martian soil analogues was tested in a setup built for this purpose, described in detail by [Garry et al. \(2006\)](#). Stainless steel sample cups were filled with 15–20 mg martian soil analogue or serpentine. The cups were placed in a brass sample holder, which can be cooled to -60°C . The samples and the cooling stage were placed in a vacuum chamber (background pressure 3×10^{-5} mbar). After evacuation the chamber was filled with 7 mbar CO_2 (Praxair, 99.996 % purity). The CO_2 was calibrated by the supplier to contain <10 ppm H_2O . If the residual gas in the system before filling with CO_2 contained only water, the upper limit of water vapour in the system during the experiments can be estimated to be 10^{-4} mbar, or 14 ppm.

Finally, a UV-grade quartz window allowed the samples to be irradiated. The irradiation source was either a short-arc xenon lamp (Sciencetech, SF150) or a deuterium lamp (Heraeus–Noblelight, DX202). The output of both lamps was measured with a UV sensor (Solartech, model 8.0) and a full range light meter (Extech, model EA30). The UV sensor is sensitive over a known profile between the wavelengths 245 and 280 nm (peak at 257 nm). The integrated output of the UV sensor was used to scale the relative spectra of both lamps as provided by their

respective manufacturers, to the absolute irradiance spectra given in figure 5.1. Also shown in figure 5.1 is the predicted irradiation spectrum of the martian surface for equatorial regions at noon-time with a dust-free atmosphere, as modelled by Patel et al. (2002). The power output of the solar simulator was optimized to agree with the Mars model of Patel et al. (2002). Integrated over the wavelength range 190–400 nm the solar simulator has an output of 30 W m^{-2} , while the Mars model yielded 32 W m^{-2} . The deuterium lamp delivered $\sim 3.4 \text{ W m}^{-2}$ in the same range.

In order to best mimic the conditions on Mars, the environmental conditions were varied in a diurnal cycle. The samples were irradiated for 12 h at room temperature, followed by cooling to -60°C without irradiation for another 12 h. This resembles a day (sol) near the martian equator, where the Sun illuminates the martian surface only during the day, when the temperature can reach as high as 20°C in equatorial regions; but UV radiation levels drop after sun set, followed by a drop in temperature, to an average of -60°C . Each experiment lasted 4 diurnal cycles, equivalent to simulate 4 martian sols.

HPLC–FD analysis

To test the degradative capacity of the martian soil analogues, the amino acid content was measured before and after 4 diurnal cycles in the Mars simulation setup. The martian soil analogues and the serpentine blank control were analysed according to the established procedure for extracting, separating, and analysing amino acids in meteorite samples (Zhao & Bada 1995,

Botta & Bada 2002, Martins et al. 2007b,a).

All glassware and ceramics were baked out before use for 3 h at 500°C . Pipette tips and Eppendorf tubes were obtained sterile from Sigma-Aldrich. All chemicals were acquired from Sigma-Aldrich, unless stated otherwise. The samples were flame-sealed in Pyrex test tubes with 1 mL water and incubated at 100°C for 24 h. After the hot water extraction, the tubes were rinsed with HPLC grade water, cracked open, and centrifuged to spin down the solid particles. 500 μL of the supernatant of each sample was transferred to a smaller test tube and dried under vacuum. For acid hydrolysis, the smaller test tube from each sample was placed in a larger Pyrex test tube, containing 1 mL 6 M HCl. The larger test tubes were flame-sealed and placed in an oven at 150°C for 3 h. The test tubes were rinsed with HPLC water, cracked open, and the smaller test tubes were taken out. The hydrolysed extracts were dried under vacuum, dissolved in 3 mL HPLC water, desalted on a AG[®] 50W-X8 cation exchange resin (100–200 mesh, BioRad), and eluted with 5 mL 2 M ammonium hydroxide (Acros Organics). The eluates were dried under vacuum and redissolved in 100 μL HPLC water. This stock solution was stored at -20°C until further use.

From the stock solution, 10 μL aliquots were added to 10 μL 0.1 M sodium borate buffer. These were dried under vacuum to remove any residual traces of ammonia. After dissolution in 20 μL HPLC water, the amino acids were derivatized by adding 5 μL OPA/NAC (o-phthaldialdehyde/N-acetyl-L-cysteine). After 1 or 15 min, the derivatization reaction was

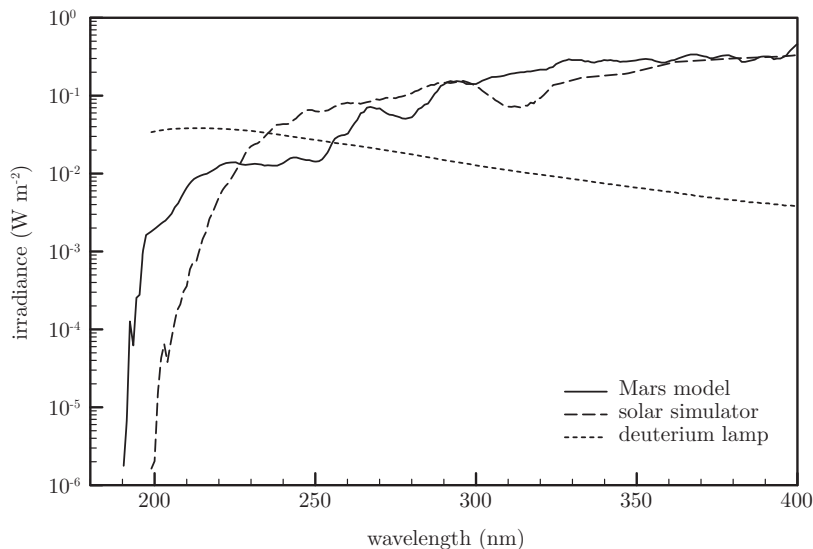


Figure 5.1

spectra of the solar simulator (dashed line) and deuterium lamp (dotted line) in the range 190–400 nm. Also shown is the predicted equatorial noon-time irradiation of the martian surface, as modelled by [Patel et al. \(2002\)](#) for a dust-free atmosphere (solid line).

quenched by adding 475 μL 50 mM sodium acetate. The derivatized amino acids were separated on a C18 reverse phase Synergi 4 μ Hydro-RP 80A column (250 $\times$ 4.6 mm, Phenomenex) and detected by UV fluorescence on a Shimadzu RF-10AXL (excitation at 340 nm, emission at 450 nm). Elution was performed by applying a binary mobile-phase gradient, where buffer A contained 50 mM sodium acetate and 4 vol% methanol, and buffer B was methanol (Biosolve Ltd.). The gradient was run at a constant flow

rate of 1 mL min^{-1} and starting with 100 % buffer A, buffer B was increased in several time steps: 0–4 min: 0 %, 4–5 min: increase to 20 %, 5–10 min: 20 %, 10–17 min: increase to 30 %, 17–27 min: increase to 50 %, 27–37 min: increase to 60 %, 37–49 min: 60 %, 49–50 min: decrease to 0 %, 50–60 min: 0 %. Amino acids were identified by comparing to known standards. The abundances of the identified amino acids were determined by the ratio of the integrated peak area to the integrated peak area of the standards, cor-

rected for the abundances found in the serpentine blank control sample.

5.4 Results

Soil properties

The pH and redox potential of the Yungay, Arequipa, and Salten Skov soils are displayed in figure 5.2(a) and (b). The closed symbols in figure 5.2 display the data directly after dissolution. After 20–30 minutes the samples were purged with nitrogen to displace the dissolved oxygen. The pH and redox potential of the purged solutions are depicted by open symbols in figure 5.2. While the pH of Flat Top Hill (Yungay) and Foot of Slope (Arequipa) samples were near neutral (7.6 and 7.3, respectively), the pH was much lower for the Salten Skov and Top of Slope (Arequipa) samples (4.6 and 4.0, respectively). Purging the solution with nitrogen did not alter the pH. A pH of 4.6 for Salten Skov is in good agreement with the pH found by Nørnberg et al. (2004). The pH of the Flat Top Hill sample (7.6) confirms the measurements from Lester et al. (2007).

The redox potential for the Salten Skov, Flat Top Hill (Yungay), and Foot of Slope (Arequipa) samples was moderate (~ 55 mV, ~ 24 mV, and ~ 0 mV, respectively) and, like the pH for those samples, was stable after 12 minutes. However, in contrast to the pH, removal of dissolved oxygen by purging with nitrogen resulted in an immediate sharp drop in redox potential. The Salten Skov potential dropped to -110 mV, while the Flat Top Hill sample dropped to -260 mV. The Foot of Slope sample followed the same curve

as the Flat Top Hill sample, but was not measured beyond 60 minutes. The Top of Slope sample showed a much higher initial redox potential (145 mV), which dropped slowly over time. It continued to drop at the same rate upon purging with nitrogen, making the drop much less sharp than for the other samples. The final potential for Top of Slope was 8 mV.

After the pH and redox potential measurements, the soil solution was centrifuged and the supernatant decanted off. The supernatants were diluted by the factor given in table 5.2, followed by conductivity and ion concentration measurements. The results for those measurements are also given in table 5.2. The results show that the Foot of Slope (Arequipa) sample has a very high salt content, 50–500 times higher than the concentrations in the sample from the top of the same slope and Flat Top Hill sample (Yungay). The conductivity is also ~ 10 times higher for the Foot of Slope sample. The Foot of Slope sample shows high concentrations of Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Cl^- , NO_2^- , and SO_4^{2-} , while the Top of Slope and Flat Top Hill samples' main constituents are Ca^{2+} and SO_4^{2-} , with the other ions available at much lower concentrations.

Laser desorption–mass spectroscopy

The Atacama desert soils were measured with LD–MS to reveal their organic content. The soil samples were analysed using straight-up laser desorption without any further sample preparation. The resulting spectra are shown in figure 5.3. In all of the LD–MS spectra a variety of high molecular weight compounds up to >1900

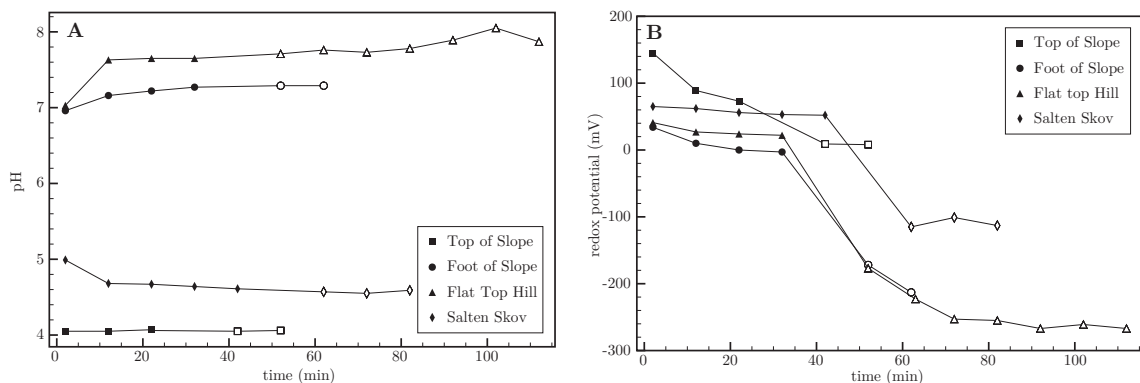


Figure 5.2

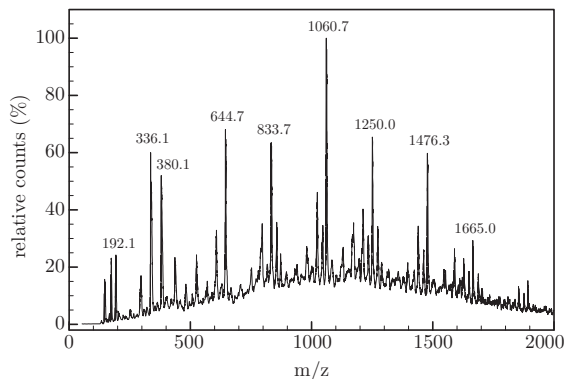
pH (a) and redox potential (b) of different soils. Top of Slope (Arequipa) soil is designated by squares, Foot of Slope (Arequipa) by circles, Flat Top Hill (Yungay) by triangles, and Salten Skov by diamonds. The closed symbols refer to the dissolved samples in air, while the open symbols display the pH of the same samples purged with nitrogen. Time is counted from the moment of dissolution, while purging with nitrogen started immediately after the last non-purged measurement (last closed symbol in the graph).

amu was observed. Peaks at 335 and 380 m/z in the Foot of Slope (Arequipa) sample (figure 5.3b) are characteristic of naphthenes that are found in some desert plants like cactus. Other peaks in the spectra separated by 12 or 14 amu (for example around 600 amu mass peaks) are assignable to the loss of hydrocarbon fragments C, CH, and CH₂ from the parent ion. Mass separations of 16 and 32 amu may represent a loss of one or two oxygen atoms (e.g. 855, 833, or 817 m/z).

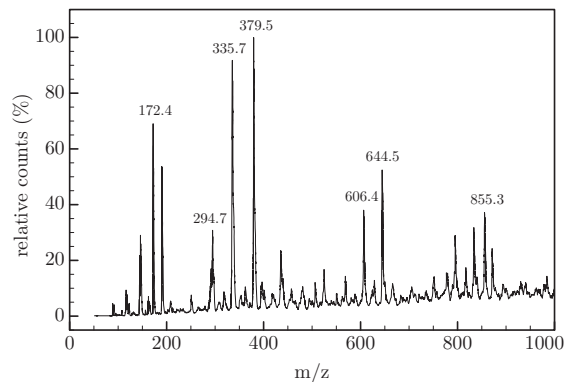
The Top of Slope soil (Arequipa, see figure 5.3a) displays peaks at 644, 834, 1060, 1250, and 1476 m/z that all exhibit multiple mass unit clusters that may arise from several compounds in-

corporating the same distribution of fatty acids (e.g. phospholipids), or alternatively may also reflect halogenation.

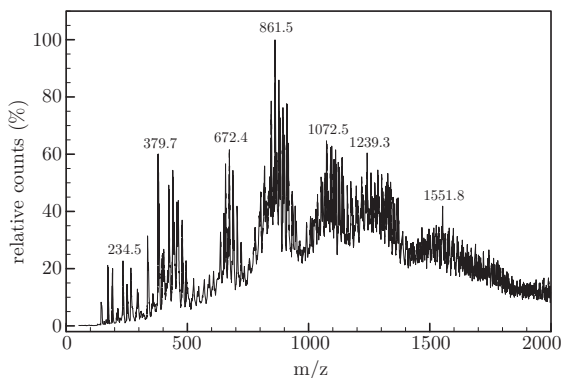
Several high molecular weight mass unit clusters are also seen in the Second Site (Yungay) sample (figure 5.3c). The peaks within each cluster are separated by 12 or 14 amu and are, again, attributed to hydrocarbon fragments as seen in the soil collected from the Foot of Slope (figure 5.3b). The Second Site sample also displays a prominent high mass envelope with peak separations of 12, 14 and 16 amu attributed to hydrocarbon and oxygen mass losses (C, CH, CH₂, and O) from the parent peak. These multiple clustered peaks may also be products of a macro-



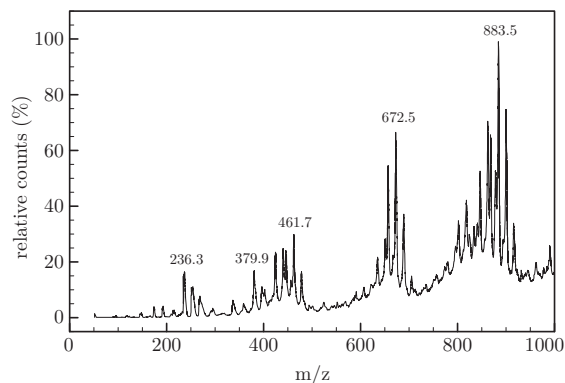
(a) Top of Slope (Arequipa)



(b) Foot of Slope (Arequipa)



(c) Second Site (Arequipa)



(d) Soil Pit (Yungay)

Figure 5.3

laser desorption–mass spectra (LD–MS) of (a) Top of Slope (Arequipa), (b) Foot of Slope (Arequipa), (c) Second Site (Arequipa), and (d) Soil Pit (Yungay). The molecules were desorbed directly from the soils with a 337 nm nitrogen laser at an intensity of 10^6 W cm^{-2} .

Table 5.2
ion concentrations in ppm and the conductivity in mS cm⁻¹ for three different soil samples. The values are corrected for dilution by the given dilution factor.

	Arequipa Top of Slope	Arequipa Foot of Slope	Yungay Flat Top Hill
cations			
Na ⁺	163	6780	36
NH ₄ ⁺	34	— ^a	<0.5
K ⁺	70	534	20
Mg ²⁺	71	1590	12
Ca ²⁺	518	827	585
anions			
Cl ⁻	93	7830	84
Br ⁻	<0.5	— ^a	<0.5
NO ₂ ⁻	<0.5	— ^a	<0.5
NO ₃ ⁻	64	5270	10
PO ₄ ³⁻	<0.5	— ^a	<0.5
SO ₄ ²⁻	1857	9878	1415
conductivity	5.074	49.39	3.736
dilution factor	14.58	9.284	11.32

^a below detection limit

molecular or kerogen-like component that is not easily desorbed and/or ionized intact resulting in the fragmentation of smaller side groups as is observed in all of the Arequipa soils.

The Soil Pit (Yungay, figure 5.3d) sample displays a mass spectrum very similar to the Second Site sample, with peaks clustered in groups around 461, 672, and 883 m/z. Compared to Sec-

ond Site, the Soil Pit has less peaks within each cluster.

An important future analytical technique to apply to these soil samples would be to perform MS/MS on some of the more prominent masses or ‘parent peaks’ observed in these spectra. This would allow for better characterization of the mass separations and may give some insight into

the possible origin of the parent peaks (e.g. bacterial, plant etc.).

Amino acids composition of Mars soil analogues

Abundances of the amino acids present in the Mars soil analogues were determined before the Mars simulation experiments were conducted. Figure 5.4a shows typical HPLC–FD chromatograms of the acid-hydrolysed hot-water extracts of the samples used in the Mars simulation experiments (Saltén Skov, Top of Slope (Arequipa), and Orgueil). Figure 5.4b shows typical HPLC–FD chromatograms of the remaining Yungay and Arequipa desert soil samples. Both figures include a chromatogram of an amino acids standard and a chromatogram of a serpentine blank. The chromatograms were scaled for clarity; the scale factors are shown in brackets in figure 5.4. The abundances of the amino acid, in parts per billion (ppb) by weight and corrected for any abundance found in the serpentine blank, are given in table 5.3 along with the associated error margins (SEM).

The desert soil samples Foot of Slope and Second Site from the Arequipa region and Soil Pit from the Yungay region all have low amino acid abundances of a few ppb, or even below the detection limit. Only the Top of Slope and Flat Top Hill samples had measurable levels of amino acids, with L-amino acids and glycine being the most abundant amino acids, while D-amino acids probably result from the racemization of microbial detritus. The measured amino acid distribution, as well as the reported abundances are

typical of the Atacama soils (Buch et al. 2006) and other desert soils (Martins et al. 2007b).

Saltén Skov has a very high amino acid content compared to the desert and meteorite samples, with abundances in the order of parts per million (ppm). L-alanine, L-glutamic acid, glycine and L-aspartic acid are the most abundant amino acids, which is a typical sign of biological activity in the soil. The abundances for Saltén Skov listed in table 5.3 agree well with the values obtained by Garry et al. (2006).

The most abundant amino acids in the Orgueil meteorite are β -alanine, glycine and β -amino-n-butyric acid (β -ABA), while all other amino acids are present at lower abundances. These results are in agreement with those previously reported by Ehrenfreund et al. (2001), Botta et al. (2007), and Martins et al. (2007b).

Mars simulation experiments

The goal of the Mars simulation experiments was to measure the stability of amino acids in Mars soil analogues after exposure to a simulated martian environment. Because the Arequipa samples contained low levels of amino acids, one sample (Top of Slope) was spiked with 4.5 ppm D-alanine before exposure to simulated Mars conditions. The Orgueil, Saltén Skov, and spiked Top of Slope samples were exposed to simulated martian conditions for 4 diurnal cycles (an equivalent of 4 martian sols). After exposure, amino acid abundances were determined using HPLC–FD as described for the non-exposed samples. The resulting abundances are shown in figure 5.5(a)–(c) (white bars), along with the abundances of the

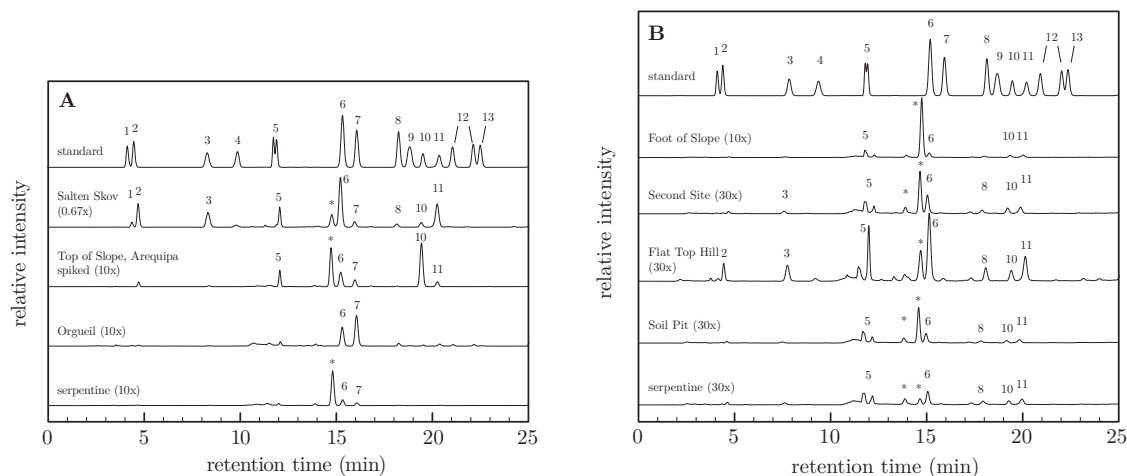


Figure 5.4

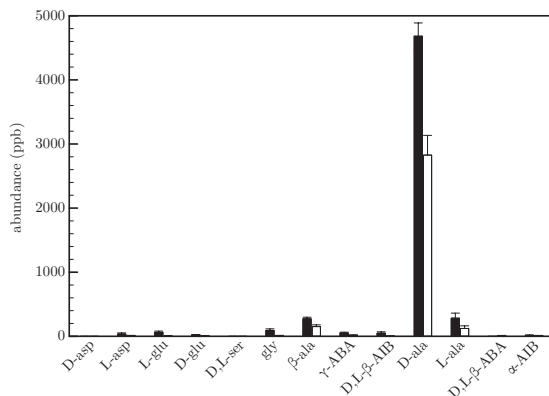
HPLC–FD chromatograms of OPA/NAC derivatized (1 min.) acid-hydrolysed hot water extracts of (a) Mars soil analogues and meteorite samples, and (b) the Arequipa and Yungay desert soil samples. The chromatograms in (a) and (b) were recorded on different days and are therefore shown in separate panels. The samples shown here have not been exposed to simulated Mars conditions. The chromatograms have been scaled for clarity, the numbers in brackets are the scale factors. The numbers in the chromatograms designate the identified peaks of 1. D-aspartic acid, 2. L-aspartic acid, 3. L-glutamic acid, 4. D-glutamic acid, 5. D- and L-serine, 6. glycine, 7. β -alanine, 8. γ -amino-n-butyric acid, 9. D- and L- β -aminoisobutyric acid, 10. D-alanine, 11. L-alanine, 12. D- and L- β -amino-n-butyric acid, and 13. α -aminoisobutyric acid. Peaks labelled with a * were not identified.

non-exposed samples (black bars). The amino acid difference between the exposed and non-exposed samples is listed in table 5.4. Negative values in table 5.4 signify an increase in abundance.

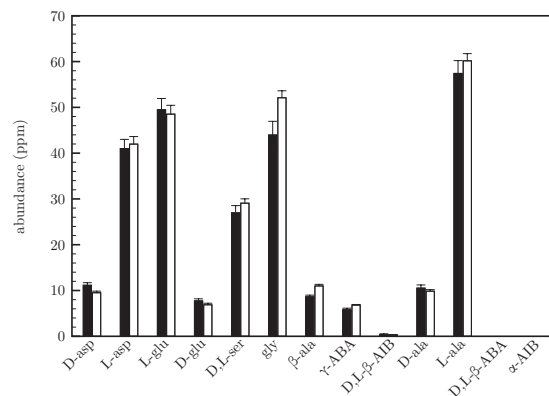
The Top of Slope sample from the Arequipa region contained very low abundances of amino acids, see figure 5.5a. Most amino acids had a

much lower abundance after exposure to the simulated Mars environment. A decrease of $\sim 85\%$ was observed for most amino acids, see table 5.4. Of the 4.5 ppm D-alanine that were added to the soil before exposure 2.7 ppm were recovered, a difference of 40 %.

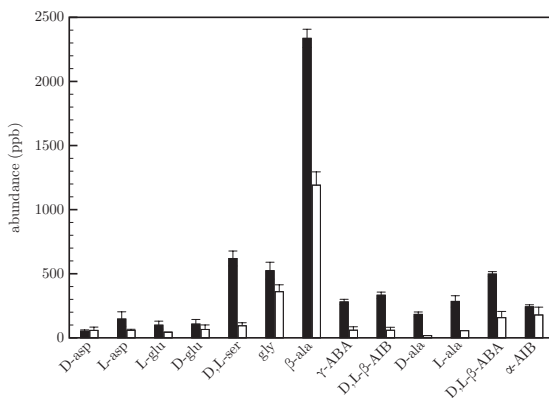
After exposure to Mars-like conditions, the Salten Skov sample still contained a high con-



(a) Top of Slope (Arequipa)



(b) Salten Skov



(c) Orgueil

Figure 5.5

Amino acid abundances in (a) Top of Slope (Arequipa) spiked with D-alanine, (b) Salten Skov, and (c) Orgueil meteorite. In each panel, the black bars give the abundance before exposure to a simulated Mars environment and the white bars after exposure. The error bars denote the standard error of the mean. Please note that the Orgueil sample was irradiated with the solar simulator, while for all other experiments a deuterium lamp was used. Spectral differences between the lamps are shown in figure 5.1.

Table 5.3

Abundances of selected amino acids as measured by HPLC–FD. The Soil Pit sample from the Yungay region did not yield any detectable amino acid abundance. The error margins are the standard error of the mean (SEM, n=7).

amino acid	Foot of Slope (ppb)	Second Site (ppb)	Flat Top Hill (ppb)	Top of Slope <i>spiked</i> (ppb)	Salten Skov (ppm)	Orgueil (ppb)
D-aspartic acid	— ^a	— ^a	13 ± 1	— ^a	11 ± 0.6	52 ± 10
L-aspartic acid	— ^a	— ^a	57 ± 17	35 ± 18	41 ± 2.0	147 ± 56
L-glutamic acid	2 ± 1	— ^a	116 ± 26	62 ± 19	49 ± 2.5	99 ± 31
D-glutamic acid	2 ± 1	— ^a	31 ± 3	20 ± 2	7.7 ± 0.4	107 ± 36
D,L-serine ^b	— ^a	— ^a	54 ± 70	— ^a	27 ± 1.6	619 ± 58
glycine	1 ± 4	— ^a	102 ± 45	91 ± 25	44 ± 3.0	524 ± 66
β-alanine	3 ± 1	1 ± 1	11 ± 2	273 ± 25	8.6 ± 0.4	2336 ± 71
γ-ABA	— ^a	— ^a	69 ± 6	52 ± 12	5.8 ± 0.3	280 ± 21
D,L-β-AIB ^{b,c}	6 ± 1	5 ± 1	— ^a	49 ± 21	0.4 ± 0.2	333 ± 24
D-alanine	21 ± 0	5 ± 4	32 ± 10	4681 ± 206	11 ± 0.7	183 ± 19
L-alanine	15 ± 1	— ^a	92 ± 35	283 ± 78	57 ± 2.8	284 ± 44
D,L-β-ABA ^c	— ^a	— ^a	— ^a	— ^a	— ^a	497 ± 19
α-AIB	— ^a	— ^a	— ^a	14 ± 8	— ^a	242 ± 16

^a below detection limit

^b Enantiomers could not be separated under the chromatographic conditions.

^c Optically pure standard not available for enantiomeric identification.

centration of amino acids, in the ppm range rather than ppb for the other Mars soil analogues, see figure 5.5b. The change in amino acid abundance in Salten Skov upon exposure varies per amino acid. Some amino acids (D-aspartic acid, L- and D-glutamic acid, D,L-β-amino-isobutyric acid (α-AIB), and D-alanine) show a decrease, while others (L-aspartic acid, D,L-serine, glycine, β-alanine, γ-amino-n-butyric acid (γ-ABA), L-alanine, D,L-β-amino-n-butyric acid (β-ABA), and α-AIB) are found at increased levels after exposure. However, for most compounds the change is small and within the error margin. Only glycine, β-alanine, and γ-ABA

display an increase in abundance after exposure that is larger than the associated error margin. The abundance change for α-AIB, β-AIB, and β-ABA is apparently large, but so is the associated error margin. This can be explained by the low abundances of these species in the original (unirradiated) Salten Skov sample.

The amino acid abundances in the Orgueil meteorite were lower after exposure to a simulated Mars environment for almost all tested amino acids. The loss in Orgueil was different per amino acid, between 31 and 90 % with most values around 65 %. The only exception is D-aspartic acid which shows an increase of 11 %,

Table 5.4
 Absolute and relative differences (Δ) in amino acid abundance in soil and meteorite samples after exposure to simulated Mars conditions, shown in figures 5.5(a)–(c). The error margins shown in this table were propagated from the error bars in figure 5.5.

amino acid	spiked Top of Slope		Salten Skov		Orgueil	
	Δ (ppb)	rel. Δ (%)	Δ (ppm)	rel. Δ (%)	Δ (ppb)	rel. Δ (%)
D-aspartic acid	— ^a	—	1.6 ± 0.7	15 ± 6.0	−6 ± 28	−11 ± 53
L-aspartic acid	27 ± 19	76 ± 65	−1.0 ± 2.6	−2.4 ± 6.4	89 ± 57	60 ± 45
L-glutamic acid	59 ± 20	96 ± 44	0.9 ± 3.1	1.9 ± 6.3	56 ± 31	57 ± 41
D-glutamic acid	17 ± 3	84 ± 15	0.9 ± 0.6	11 ± 7.3	42 ± 51	39 ± 36
D,L-serine ^b	— ^a	—	−2.1 ± 1.8	−7.8 ± 6.8	525 ± 63	85 ± 13
glycine	83 ± 25	90 ± 37	−8.1 ± 3.4	−18 ± 7.7	164 ± 85	31 ± 17
β-alanine	121 ± 37	44 ± 14	−2.3 ± 0.5	−27 ± 5.9	1145 ± 126	49 ± 6
γ-ABA	36 ± 15	71 ± 33	−1.0 ± 0.4	−17 ± 6.5	220 ± 34	79 ± 13
D,L-β-AIB ^{b,c}	47 ± 21	97 ± 59	0.1 ± 0.2	25 ± 50	274 ± 34	82 ± 12
D-alanine	1854 ± 370	40 ± 8	0.7 ± 0.8	6.4 ± 7.3	165 ± 19	90 ± 14
L-alanine	161 ± 87	57 ± 34	−2.7 ± 3.2	−4.8 ± 5.6	228 ± 44	80 ± 20
D,L-β-ABA ^c	— ^a	—	— ^a	—	340 ± 52	68 ± 11
α-AIB	5 ± 9	35 ± 65	— ^a	—	63 ± 63	26 ± 26

^a below detection limit
^b Enantiomers could not be separated under the chromatographic conditions.
^c Optically pure standard not available for enantiomeric identification.

but which also shows a large associated error bar.

5.5 Discussion

Mars soil analogues

Parts of the Atacama desert are among the driest places on Earth. For this reason, the soils in these desert regions have been used as an analogue for the regolith of Mars (Navarro-González et al. 2003). Besides being extremely dry, the

martian regolith is also depleted in most organic compounds above ppb levels, as inferred from the Viking data (Biemann et al. 1976, 1977, Biemann 2007). From the results in table 5.3 it is clear that the Arequipa and Yungay soils are largely devoid of amino acids. This makes them ideal to use in Mars simulation experiments, especially in experiments where the stability of amino acids – and possibly other organics– are investigated.

The two samples named Top of Slope and Foot of Slope were taken from the same slope

in the Arequipa region, only metres apart. The data in figure 5.2 and table 5.2 show a remarkable difference in chemical and physical properties between the two samples. The Top of Slope sample has a low pH (4.0) and high redox potential (150 mV) compared to the neutral pH (7.1) and redox potential (10 mV) of the Foot of Slope. The ion concentrations in the Foot of Slope sample are 10–80 times higher than in the Top of Slope sample. The organic content of the Top and Foot of Slope samples are similar (figure 5.3). Both display peaks at 336, 380, 644, 833, and 855, but the relative intensities between the peaks are different for each samples. However, when the results of the pH, redox potential, and ion concentrations measurements of the Top of Slope sample are compared with the Flat Top Hill sample from the Yungay region—800 km south-east of the Arequipa region—they are very similar. The similarity extends to the amino acid abundances, which are low but measurable for both the Top of Slope (Arequipa) and Flat Top Hill (Yungay) samples. The abundances for those samples are in the range of 10–200 ppb. For the Arequipa samples Foot of Slope and Second Site and the Yungay sample Soil Pit, the amino acid abundances are mostly below the detection limit. Overall, the Arequipa and Yungay soils appear highly heterogeneous and patchy.

Amino acid stability

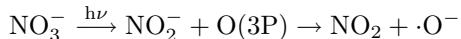
We have tested the stability of amino acids in the Top of Slope soil sample from the Arequipa region. Because of the low abundance of amino acids in that sample, the soil was spiked with D-

alanine before exposure to a simulated Mars environment. Other samples exposed to the simulated Mars environment had a detectable level of amino acids and did not require spiking. The results in figure 5.5 and table 5.4 show a decrease in amino acid abundance for the Arequipa soil and Orgueil meteorite, but not for the Salten Skov sample. A solar simulator lamp was used for the Orgueil experiment, while the Top of Soil and Salten Skov samples were irradiated with a deuterium lamp. The spectral differences are shown in figure 5.1. Degradation of amino acids directly by UV radiation is possible (ten Kate et al. 2005), but UV radiation will be attenuated by the first layer of soil grains it encounters. Direct degradation by UV radiation would therefore only photolyse the amino acids that are located on top of the soil grains and a destruction of only a few % would be the result. However, figure 5.5 and table 5.4 show that the loss of amino acid is much larger for the Orgueil and Arequipa samples, which implies that additional mechanisms for degradation are active.

The Mars simulation experiments were conducted in a diurnal cyclic mode, starting with 12 h of irradiation, followed by 12 h of cooling to -60°C . This cycle was repeated 4 times. The Mars simulation chamber contained <14 ppm water during the experiment, see section 5.3. This is comparable to the lower end of the range in which water vapour varies in the martian atmosphere, between 10–1000 ppm (Encrenaz et al. 2004). Upon cooling of the samples, water freezes from the CO_2 atmosphere onto the samples.

Table 5.2 shows that the Top of Soil sample from the Arequipa desert contains 64 ppm NO_3^- .

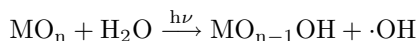
When the samples are irradiated, photoactivation of nitrate can occur.



When water condenses or absorbs onto the samples, the oxide radical can react with water to form an OH radical.



Similar reactions can occur with metal oxides present in the soil.



In combination with iron bearing minerals, a photon-enhanced Fenton-type (photo-Fenton) reaction can occur. Recent research has shown that OH radicals and H_2O_2 can also be formed by mechanical pulverization of basaltic rocks, i.e. by impact and aeolian abrasion (Hurowitz et al. 2007). Radicals produced in the above reactions can oxidize the amino acids in the soil, leading to their destruction. The production of radicals and the linked degradation of amino acids is therefore dependent on the mineralogical composition of the soil.

Mineralogy

The loss of amino acid in the Top of Slope sample from the Arequipa region is $\sim 40\%$, while for Salten Skov, exposed to the same conditions, the loss is marginal. For Orgueil the loss after exposure is $\sim 80\%$, but a solar simulator was used to irradiate the Orgueil sample instead of the deuterium lamp. The Orgueil meteorite has previously been shown to contain the hydrous phyllosilicate minerals serpentine and saponite, and

ferrihydrite as bulk material (Tomeoka & Buseck 1988, Miyamoto & Zolensky 1994). Salten Skov is a very fine powder, consisting of the silt and clay fractions of the soil, with grain sizes $< 63\ \mu\text{m}$. It is mainly composed of goethite, with additions of hematite and maghemite (12.8 wt% and 5.5 wt%, respectively). Although the mineralogy for the Top of Slope sample is not known, we have shown that it contains calcium and sulphate ions, see table 5.2. The Top of Soil was sampled from the top layer, which included a duricrust. Duricrust can contain high amounts of gypsum, which agrees with the high calcium sulphate concentration we found. Many of the minerals identified in these Mars regolith analogues, including gypsum, have recently been found on Mars. Chevrier & Mathé (2007) give a comprehensive overview of the mineralogical details found by the Mars Exploration Rovers and MarsExpress, which include phyllosilicates, serpentine, sulphate minerals, goethite, hematite, and widespread anhydrous ferric oxides. Candidate minerals for the anhydrous ferric oxides are nanocrystalline hematite and maghemite.

Destruction of amino acids —and organics in general— is influenced by the mineralogical composition of the soil. The relative stability of amino acids in the Salten Skov sample compared to the other samples (Arequipa and Orgueil) can be attributed to a mineralogical composition of Salten Skov that stabilizes organic compounds on its mineral surfaces. Salten Skov is a clay-rich material and clays have the ability to sequester organics, preventing their destruction.

5.6 Conclusions

We have obtained several soil samples from different regions of the Atacama desert, and measured the physical and chemical properties of those samples including pH, redox potential, ion concentrations, conductivity, the organic content, and more specifically amino acid abundance. The results show that samples which are located close together, separated by only a few metres, can vary strongly in chemical and physical properties.

HPLC–FD measurement of hot-water extracts of the Arequipa and Yungay soil samples show that they are largely devoid of amino acids, although there is variation between the samples. Three desert soil samples (two Arequipa and one Yungay) have amino acid abundances near or below the detection limit, while two others (an Arequipa and an Yungay sample) have abundances in the 10–200 ppb range. This is still a factor 100 lower than the precipitated sediment of Salten Skov, which has amino acids in the ppm range.

One of the Arequipa desert samples (Top of Slope), the Salten Skov sample, and a sample of the Orgueil meteorite were exposed to a simulated Mars environment. In this simulation the samples were exposed to 12 h UV irradiation, followed by 12 h of cooling to -60°C . This was repeated 4 times to simulate 4 martian diurnal cycles. After the exposure, the amino acid abundances were measured and compared to the abundances of the non-exposed samples. The Orgueil meteorite and Arequipa desert soil showed a lower amino acid abundance after exposure to the simulated Mars environment, whereas

Salten Skov did not show a significant change of amino acid abundance. The Salten Skov sediment seems to protect the amino acids from destruction. The mineralogical composition of the soil strongly influences the stability (or destruction) of the amino acids. It is therefore important that future missions to Mars consider the complex interactions between organic compounds and the mineralogy of a proposed landing site, to increase the chance of finding organic molecules on Mars.

Acknowledgements

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Six

**Survival and death of the haloarchaeon *Natronorubrum* sp.
strain HG-1 in simulated early Earth and martian environments**

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Abstract

Halophilic archaea are of interest to astrobiology due to their survival capabilities in desiccated and high salt environments. The detection of extraterrestrial salts in meteorites and the possibility of former salty pools on Mars stimulated investigations into the response of haloarchaea to Martian conditions. *Natronorubrum* sp. strain HG-1 is an extremely halophilic archaeon with unusual metabolic pathways, since it grows on acetate and is stimulated by tetrathionate (Sorokin et al. 2005). We investigated the effects of high and low temperature as well as desiccation on *Natronorubrum* strain HG-1. Growth curves following exposure to extreme conditions showed that the viability of the strain in liquid medium was not affected by a 24 h storage at 4 or -20°C . Lag phases of cultures were increased when samples were desiccated and/or stored at low temperatures. Samples stored at 70°C did not show any growth at all. We exposed *Natronorubrum* strain HG-1 to ultraviolet (UV) radiation, similar to the levels currently prevalent on Mars. Samples irradiated with UV radiation for only 30 min showed a survival rate of $<10^{-4}\%$, while samples irradiated for 24 h did not show any growth. Samples desiccated onto soil from the Atacama desert showed no growth after rehydration. From the results it can be concluded that *Natronorubrum* strain HG-1 cannot survive for more than a few minutes when exposed to UV radiation equivalent to that at the martian equator during local noon. Even when protected from UV radiation, viability may be impaired by a combination of desiccation and low temperature. In addition, the lethal effect of exposure to 70°C indicates that this strain could not have existed during the Precambrian era when ocean temperatures were thought to be high.

6.1 Introduction

Halophiles are a class of Archaea that not only tolerate, but also thrive in environments with high salt concentrations up to saturated solutions. These salt-tolerant organisms are thought to have evolved early in the Earth's history (Oren 2002). Geological formations that are millions of years old, still contain viable halophilic archaea and bacteria (e.g. 290–206 million years ago, the Permian-Triassic era Norton & Grant 1988, Stan-Lotter et al. 1999, Vreeland et al. 2000, McGenity et al. 2000). Mancinelli et al. (1998) have shown that osmophilic microbes *Synechococcus* Negeli

and *Haloarcula*-G survived a two week exposure to the space environment while in Earth orbit aboard the Biopan facility.

A possible connection between the origin of life on Earth and the ability of microorganisms to grow at high salt concentrations has been described by Dundas (1998), who suggested that microvesicles may form easily under the isohaline conditions of hypersaline brines. Litchfield (1998) concluded that the ability of halophilic archaea to survive within low water-activity environments, such as evaporites, and their requirement for elevated salt concentrations make them model organisms for life on Mars. Mars is

thought to have had liquid water present at its surface for a geologically long period (Squyres et al. 2004). The progressive desiccation of the surface in a later phase led to an increase in the salt content of the remaining bodies of water. Since on Earth microbial life is thriving in similar environments, mechanisms for tolerance of high salt concentrations could be envisaged to have evolved in potential martian organisms.

The effects of simulated martian environmental stresses have been tested on a few microorganisms including cyanobacterium *Chroococcidiopsis* sp. 029 (Cockell et al. 2005), several *Bacillus* species (Schuerger et al. 2006), *Escherichia coli* and *Deinococcus radiodurans* (Diaz & Schulze-Makuch 2006), and *Halobacterium salinarum* NRC-1 and *Haloarcula*-G (Mancinelli et al. 2004). In those experiments the response to high salt concentration, low temperature, desiccation and UV irradiation was studied. Rettberg et al. (2004) studied the survival of *Bacillus subtilis* spores mixed with thin layers of Mars soil simulant aboard the Biopan facility. They found that thin layers of clay, rock, or meteorite could only successfully protect spores against solar radiation when in direct contact.

The halophilic archaeon *Natronorubrum* sp. strain HG-1 was cultivated from hypersaline lakes in the Kulunda steppe, Altai, Russia by Sorokin et al. (2005). It displays typical haloarchaeal features, including pink to red colour due to carotenoids and bacterioruberin. These pigments were suggested to have a protective effect against damage by UV radiation in the related *Halobacterium salinarum* (Shahmohammadi et al. 1998). In contrast to most other

haloarchaea, *Natronorubrum* sp. strain HG-1 uses simple compounds (acetate) as a carbon source and is able to reduce tetrathionate. These features make this organism well-suited to study the responses to environmental stresses with defined growth media.

In this paper, we report the results of survival experiments, where *Natronorubrum* strain HG-1 was exposed to similar levels of UV radiation as found on the surface of Mars. In addition, we tested the survival of *Natronorubrum* strain HG-1 stored at high and low temperatures. The cells were subjected to these conditions either in suspension or after desiccation, i.e. while embedded in salt crystals. We also tested the survival of desiccated cultures of *Natronorubrum* strain HG-1 mixed with soil from the Atacama desert, as a Mars soil analogue. The results of these experiments are discussed and the chance for survival of *Natronorubrum* strain HG-1 on Mars is evaluated.

6.2 Experimental

Strain and culture conditions

Natronorubrum sp. strain HG-1 was isolated and described by Sorokin et al. (2005). *Natronorubrum* strain HG-1 cultures were grown by inoculating 10 mL fresh medium in 60 mL glass bottles (4.3 cm diameter, with not fully tightened screw caps) with 0.2 mL of a previous culture. After incubating for 7 days at 37 °C the cultures reached maximum density. The growth medium included 240 g L⁻¹ NaCl, 2 g L⁻¹ K₂HPO₄, and 0.5 g L⁻¹ (NH₄)₂SO₄. The pH was adjusted to

7.3 before sterilisation by heating for 20 minutes at 120 °C. After sterilisation, the medium was supplemented with 2 mM MgCl₂, 10 mM sodium acetate, 0.05 g L⁻¹ yeast extract, and 1 mL L⁻¹ of trace elements solution (Pfennig & Lippert 1966).

Growth was monitored by measuring the optical density (OD) at 600 nm on a double-beam UV/Vis spectrometer (Cary 3 Bio, Varian). The OD of the cells was correlated to absolute numbers by cell counting with an optical microscope. We found that an OD of 0.033 corresponded to 1.5×10^8 cells mL⁻¹ or 5×10^9 cells mL⁻¹ OD⁻¹. The OD was assumed to increase in proportion with the number of cells in suspension up to an OD of ~0.3. The maximum cell density of a culture in stationary phase reached an OD of ~0.56.

Atacama desert soil

Soil obtained from the Atacama desert in Chile (Flat Top Hill, S25°29'50.9" W69°50'22.5") was used as a Mars regolith analogue. This soil was previously used as a Mars soil analogue, to determine the stability of intrinsic amino acids under simulated Mars condition (Peeters et al. 2007). To test for the presence of halophilic microorganisms in the Atacama soil that could interfere with our experiments, we added 2 g of soil to 5 mL of medium and incubated this suspension at 37 °C. No growth was observed after several weeks.

Sample preparation

The primary culture from which all samples were derived was grown to stationary phase (OD =

0.51 or 2.6×10^9 cells mL⁻¹). 3 mL of this primary culture was added to 42 mL fresh medium, yielding 45 mL of stock culture with OD = 0.034. This stock culture was divided into 18 sterile plastic Petri dishes (Greiner Bio-One, 3.5 cm diameter, with spacer in lid) and 10 glass bottles, 1 mL per Petri dish or bottle, yielding 28 samples of 1.5×10^8 cells. The samples were then divided into two groups: 10 wet samples and 18 dry samples. The wet samples, in the bottles, were used without further treatment and directly subjected to their test environment, see section 6.2. The dry samples, in the Petri dishes, were placed without lids in a 40 °C oven. After 4 h, the samples were dry as was apparent from the formation of salt crystals and the absence of liquid. During the drying period, the weight of the samples was frequently measured and the samples were considered dry when the weight did not show any further decrease. All samples, both wet and dry were then placed in the environment to be tested.

Additional samples for the soil experiment were prepared by transferring 1 mL of stationary cells (OD = 0.51) to 6 empty Petri dishes and 4 to Petri dishes each containing 1.0 g Atacama soil. The Petri dishes with samples were placed in a 37 °C oven for 24 h with open lids to dry the samples. Two samples without soil were wrapped in Parafilm to prevent evaporation of the medium and were used as liquid controls.

Conditions of exposure

Each condition (except exposure to the deuterium lamp, see below) was tested with three samples, using exposure times of 24 h. Wet sam-

Table 6.1

Overview of the environments to which samples of *Natronorubrum* strain HG-1 were exposed. Growth curves were measured for the top half of the table, while the bottom half was measured with Most Probable Number (MPN) method.

sample	state	environment
control	liquid	—
wet blank	liquid	4 °C, dark, 24 h
wet hot	liquid	70 °C, dark, 24 h
wet cold	liquid	−20 °C, dark, 24 h
dry cold	dry	−20 °C, dark, 24 h
dry solar simulator	dry	18 °C, solar simulator, 24 h
dry deuterium	dry	18 °C, deuterium lamp, 24 h
dry blank batch 1	dry	18 °C, ambient light, 24 h
dry blank batch 2	dry	18 °C, ambient light, 48 h
control	liquid	—
dry	dry	—
dry with soil	dry	Atacama soil
dry irradiated	dry	solar simulator , 30 min
soil irradiated	dry	Atacama soil, solar simulator, 30 min

ples were stored in the dark at −20 °C, 70 °C, or 4 °C. Desiccated samples were stored at −20 °C (wrapped in Parafilm to prevent the attraction of moisture) or at room temperature. The desiccated samples were irradiated at room temperature with either a short-arc xenon solar simulator (ScienceTech, SF150) or a deuterium discharge lamp (Heraeus-Noblelight, DX 202). The solar simulator was used without the optional AM0 filter, to use the full output of the lamp down to 200 nm. The intensity of the light was measured for both lamps in the UV and visible regions, us-

ing a Solartech UV-C sensor (model 8.0) and an Extech light meter (EA30). The Solartech sensor measures irradiance over a known sensitivity profile over the wavelength range 240–280 nm with a peak at 257 nm. The Extech sensor is a broadband optical sensor and was used to ensure that the solar simulator’s output was stable for the duration of the experiment. Relative intensity spectra of both the solar simulator and the deuterium lamps were provided by the respective manufacturers of the lamps. The output of the Solartech UV sensor integrated over the 240–

280 nm wavelength range, was used to scale the spectra of the lamps. The resulting spectra are shown in figure 6.1, along with a the surface illumination spectrum of an equatorial region on Mars for a dust-free atmosphere at noon-time, as modelled by Patel et al. (2002). With the control box of the lamp set to 8.0 A constant current, the solar simulator produced an output of 30 W m⁻² in the wavelength range 200–400 nm. Both lamps had an output of 3.4 W m⁻².

The solar simulator produced a beam spot with a diameter of 11.3 cm at a distance of 31.0 cm. This was large enough to irradiate 3 Petri dishes simultaneously. The deuterium lamp produced a spot with a diameter of 4 cm at a distance of 8.5 cm. Since the deuterium lamp could be used for irradiation of only 1 sample at a time, two identical lamps were used simultaneously. The irradiation experiments were repeated after 24 h; the two experiments are referred to as batch 1 and 2. The samples of batch 2 were prepared at the same time as batch 1 and were stored at room temperature during the intervening 24 h period. A set of control samples was also included in the two batches.

Growth curves

After the 24 h storage period, the samples were allowed to equilibrate to room temperature for 1 h. The desiccated samples were rehydrated by adding 1 mL sterile demineralized water and 4 mL medium to the Petri dishes. Then, both the rehydrated samples and the stored cell suspension samples were transferred to sterile 60 mL bottles. The Petri dishes were then washed three

times with 5 mL medium. The medium used for washing was added to the rest in the bottles, resulting in a final volume of 20 mL of culture. These cultures were incubated in a water bath at 37 °C, while shaking at 40 rpm. A control culture was included by adding 1 mL of the original primary culture from which the samples were derived, to 19 mL of fresh medium. The start of the incubation was noted as $t = 0$. The growth of the samples and the control was followed in time by taking 1 mL aliquots every few hours and measuring the OD.

Analysis of the data

The OD values were converted into cell density, using a conversion factor of 5×10^9 cells mL⁻¹ OD⁻¹ obtained from optical cell counting (see above). The resulting data points were fitted with a model based on the Gompertz equation, which describes bacterial growth using three parameters: the maximum growth rate ($\mu_{\max}$), the lag (L), and an asymptotic value (A) for the maximum cell density in the stationary phase (Zwietering et al. 1990).

$$\ln \left(\frac{N(t)}{N_0} \right) = A \exp \left[\frac{\mu_{\max} e}{A} (L - t) + 1 \right] \quad (6.1)$$

In equation 6.1, $N(t)$ is the cell density at time t , N_0 is the cell density at time $t = 0$, and e is the base of the natural log. Since N_0 is unknown, and is in fact the number we are looking for, equation 6.1 was expressed using $A = \ln(N_{\max}/N_0)$, with $N_{\max}$ the maximum cell density, see equation 6.2. N_0 was allowed to vary as a fitting parameter besides $N_{\max}$, $\mu_{\max}$, and L . N_0

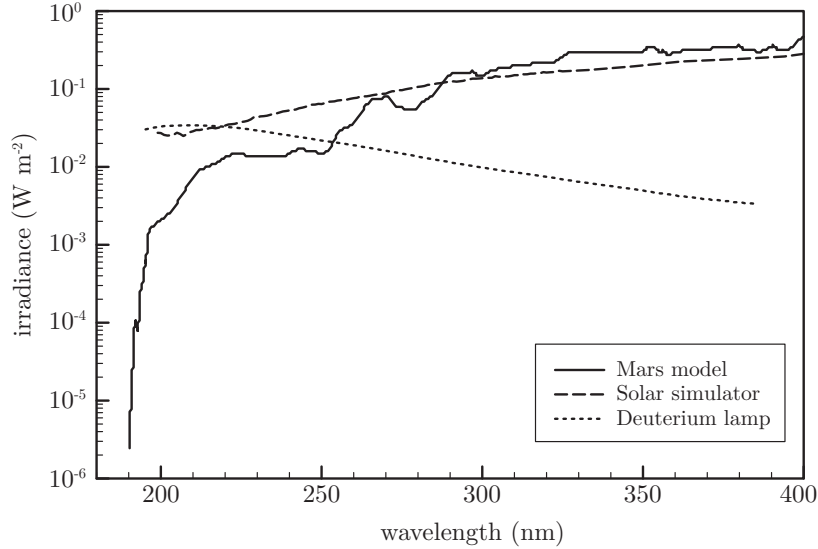


Figure 6.1

UV spectrum of the solar simulator (dashed line), the deuterium lamp (dotted line), and the lighting spectrum of Mars (solid line) in the range 190–400 nm. The Mars spectrum is the predicted equatorial noon-time irradiation of the martian surface, as modelled by [Patel et al. \(2002\)](#) for a dust-free atmosphere. The solar simulator was used without any filters, giving the full output of the lamp down to 200 nm.

is expected to lie between 0, when none of the cells survive, and 7.5×10^6 cells mL^{-1} , when all cells survive. This is below the detection limit of $\sim 1 \times 10^7$ cells mL^{-1} . Extrapolating N_o to a small number below the detection limit causes N_o to have a large uncertainty interval. Because N_o can

not easily be determined from the data, the results will be discussed based on differences in lag (L) and maximum specific growth rate ($\mu_{\max}$).

The lag phase (referred to as lag) is the time it takes for a newly inoculated culture to enter the logarithmic growth phase. It is defined as the

$$N(t) = N_o \exp \left\{ \ln \left(\frac{N_{\max}}{N_o} \right) \exp \left[- \exp \left(\frac{\mu_{\max} e}{\ln(N_{\max}/N_o)} (L - t) + 1 \right) \right] \right\} \quad (6.2)$$

X-intercept of the tangent to the inflection point, while $\mu_{\max}$ is equal to the slope of the curve in the inflection point (see figure 6.2). Lag is a property of the total cell culture and does not directly relate to the lag of the individual cells¹. The lag and the other fitting parameters $N_{\max}$, N_o , and $\mu_{\max}$ are related to the inflection point, the point at which the maximum specific growth rate ($\mu_{\max}$) occurs (figure 6.2). Combining equations 4, 9, and 10 from [Zwietering et al. \(1990\)](#) and replacing A with $\ln(N_{\max}/N_o)$, lead to the following expression for t_i , the time at which the inflection point occurs.

$$t_i = \frac{\ln(N_{\max}/N_o)}{\mu_{\max}e} + L \quad (6.3)$$

For two curves with the same inflection point, an increase in $\mu_{\max}$ will lead to an increase in L . This change is marked by arrow 1 in figure 6.2. Alternatively, when two curves have a different $\mu_{\max}$, and also a different inflection point, then not all the change in L can be attributed to an alteration of $\mu_{\max}$ and an additional growth retarding mechanism must be in effect, depicted by arrow 2 in figure 6.2. Therefore, both the growth rate and the inflection point can contribute to the lag.

Most Probable Number analysis

The liquid samples of the soil experiment were transferred to a bottle containing 5 mL medium. The Petri dish was washed twice with 2 mL

medium, which was also added to the bottle, resulting in a total culture volume of 10 mL. The dried samples were rehydrated by adding 2 mL medium and shaking on a flat-bed rotary shaker for 15 min at 110 rpm. The dissolved cell suspension was then transferred to a bottle containing 5 mL medium. The Petri dish was washed once with 2 mL medium and once with 1 mL medium. For the samples mixed with soil the same procedure was followed. A small amount of soil being transferred from the Petri dish to the bottle could not be avoided.

At this stage, all samples were in a 10^{-1} dilution. The samples were then diluted further to 10^{-2} and 10^{-3} . From the 10^{-3} dilution step the dilution series was continued in steps of 2 for another 10 dilutions (viz. 5×10^{-4} , 2.5×10^{-4} , 1.3×10^{-4} ... 1.0×10^{-6}). All dilutions were incubated in a 37 °C water bath without shaking for two weeks and were checked regularly for growth. The cell concentration in the original sample was calculated from the bottle with the highest dilution that still showed cell growth.

6.3 Results and discussion

Samples of *Natronorubrum* strain HG-1 were exposed to high (70 °C) and low (−20 °C) temperature in suspension in growth medium. Dried cultures of *Natronorubrum* strain HG-1 were exposed to low (−20 °C) temperature, light from a solar simulator or a deuterium lamp. After exposure, the cultures were rehydrated and al-

¹Each cell, provided that it is still viable, goes into growth phase at a different time. This leads to a distribution of cell lags, but the shape of the distribution is unknown, preventing the determination of N_o from the lag (see also [Baranyi 1998](#), [Robinson et al. 2001](#)).

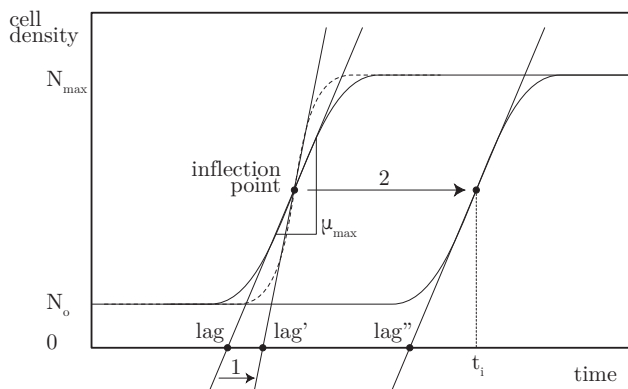


Figure 6.2

A simulated growth curve, to show the parameters that determine the shape of the curve. N_o is the cell density at time $t=0$ and $N_{\max}$ is the maximum cell density. $\mu_{\max}$ is the maximum specific growth rate, which is equal to the slope of the curve in the inflection point. Lag is defined as the X-axis intercept of the tangent to the curve in the inflection point and depends therefore on $\mu_{\max}$. If the growth rate ($\mu_{\max}$) increases, the lag will increase (to lag') by rotation around the inflection point, as depicted by arrow 1. However, if the time at which the inflection point occurs (t_i) increases, the lag will also increase (to lag''), arrow 2). Therefore, both the growth rate and the inflection point can contribute to the total lag. The relation between the lag, the inflection point and the growth rate is given by equation 6.3.

lowed to grow. The resulting growth curves are shown in figure 6.3. A summary of the results, relative to the control experiment is given in table 6.3. The modified Gompertz equation was fitted to the growth curve data. No fitting was applied to the high temperature and the irradiated samples (WH, DX and DD in figure 6.3, (a) and (c)), because those samples did not show any growth for the duration of the experiment. Some curves in figure 6.3 showed a poor fit towards the stationary phase. The Gompertz equation (as indeed many other growth models) does not account for cell death and a decrease in population size that occurs after the maximum cell density has been reached. Some curves showed a drop in OD (traces labelled WB and WC in figure 6.3(a) and (b)) right after the maximum was reached. Coagulation of the cells into clumps might explain the drop in OD, but it does not

explain why other samples (control and DC) did not show this effect. The parameters that resulted from the fitting procedure were used to calculate the inflection point, according to equation 6.3. The results are shown in table 6.2.

High temperature

Sorokin et al. (2005) found that *Natronorubrum* strain HG-1 does not grow above 46 °C. No further tests have been performed to determine viability at higher temperatures. From the data points in 6.3a (growth curve labelled WH) it is clear that *Natronorubrum* strain HG-1 does not survive a 24 h period at 70 °C, since no growth was measurable following 120 h of incubation. Recent data of oxygen and silicon isotope ratios in cherts indicate that the temperature of the Earth's oceans ~3.5 billion years ago was ~70 °C (Knauth 2005, Robert & Chauss-

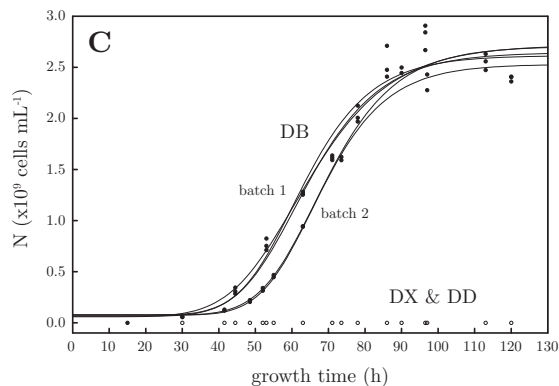
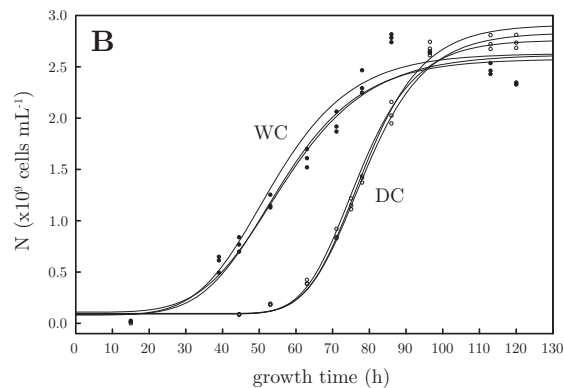
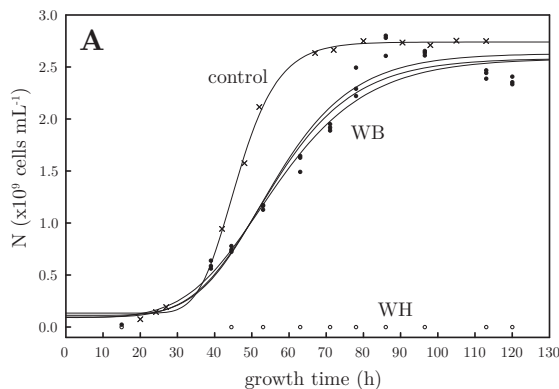


Figure 6.3

Growth curves with regression lines. The data points in this figure reflect the cell densities over time. The lines represent the curves resulting from fitting equation 6.2 to the data points. (a) Shows the control sample (crosses), the wet hot samples (WH, open circles), and wet blanks (WB, solid dots). (b) Shows the wet cold samples (WC, solid dots) and dry cold samples (DC, open circles), while (c) shows the dry solar simulator and dry deuterium samples (DX & DD, open circles) and dry blanks (DB, solid dots). The dry blanks designated batch 1 were stored in desiccated state for 24 h, those labelled batch 2 were stored 48 h.

don 2006). Around 2 billion years ago, the ocean temperature started to drop until it reached ~ 30 °C approximately 1.2 billion years ago. It remained at that temperature with some fluctuations for around 400 million years. According to the ocean temperature scale of Robert & Chausidon (2006), *Natronorubrum* strain HG-1 could have existed only later than 1.7 Gyr ago, when the temperature of the Earth's oceans dropped below 45 °C. Furthermore, *Natronorubrum* cells lyse in water with low ionic strength, e.g. less than 1.5–2 M NaCl (Grant et al. 2001), and are inactivated by such conditions in the laboratory. Unless special structures (spores, cysts, or other morphological cellular adaptations) are formed as considered by Grant et al. (1998), they would not survive in today's oceans (~ 0.6 M NaCl), nor in the early Earth's oceans where the salinity was 1.5–2 times higher (Knauth 1998). Approximately 2.5 Gyr ago, large scale salt deposition by evaporation and brine sequestration into groundwater pockets led to the lowering of ocean salinity, which continued until ~ 1 Gyr ago (Knauth 1998). In the evaporites and pockets of brine storage the salt concentration would have been high enough for *Natronorubrum* strain HG-1 to survive, but only after ~ 1.7 Gyr ago, due to the temperature limitation.

Low temperature

Figure 6.3a shows a comparison between the wet blanks (WB) and a control sample. The wet blank samples were stored at 4 °C for 24 h, while the control sample was inoculated with 1 mL of an exponentially growing culture at 37 °C. This

resulted in the control having a maximum specific growth rate ($\mu_{\max}$) almost double that of the stored wet blank samples. A reason for this higher growth rate could be the enrichment of the medium with growth stimulating factors. If *Natronorubrum* strain HG-1 cells excrete growth-stimulating factors into the surrounding medium, neighbouring cells can be stimulated into cell division. For a cell culture in logarithmic growth phase, the medium would be enriched with these growth-stimulating factors. When the control sample was inoculated, these growth-stimulating factors would have been transferred along with the cells, thereby enriching the fresh medium and enhancing the growth rate compared to cells without enriched medium. However, it is not known how medium enrichment influences the growth rate of *Natronorubrum* strain HG-1. Besides a doubling in growth rate for the control culture compared to the wet blanks, also the lag increased. But since the inflection point (t_i) for the wet blanks and the control occur at the same time, any difference in lag can be attributed to a change in growth rate (cf. equation 6.3).

The same inflection point was also found for the wet cold (WC) samples that were stored at -20 °C. Besides the inflection point, all other fitting parameters of the wet cold samples were comparable to the wet blanks, which shows that lowering the storage temperature from 4 to -20 °C for 24 h has a negligible effect. The wet cold samples were not frozen at -20 °C, due to the high salt content. Cell damage under cold conditions is usually attributed to the formation of ice crystals in the cell, which damage the cell's membranes. While the high salt concentration pre-

Table 6.2
Parameters resulting from fitting equation 6.2 to the data shown in figure 6.3. The error limits are the propagated standard errors of the fits. Also shown is the time at which the inflection point occurs, t_i , calculated from the other fitting parameters using equation 6.3.

experiment	$N_{\max}$ ($\times 10^9$ cells mL^{-1})	N_o ($\times 10^8$ cells mL^{-1})		
control	2.74 ± 0.01	1.3 ± 0.2		
wet blanks	2.60 ± 0.08	1.0 ± 1.2		
wet cold	2.61 ± 0.08	0.93 ± 1.3		
dry cold	2.83 ± 0.04	0.95 ± 0.30		
dry blanks	2.64 ± 0.07	0.71 ± 0.70		
dry blanks batch 1	2.66 ± 0.09	0.68 ± 1.1		
dry blanks batch 2	2.62 ± 0.09	0.77 ± 0.47		
experiment	$\mu_{\max}$ (cells mL^{-1} h^{-1})	L (h)	t_i (h)	
control	0.16 ± 0.01	29 ± 1.2	37 ± 2.2	
wet blanks	0.085 ± 0.03	21 ± 9.7	35 ± 21	
wet cold	0.089 ± 0.03	20 ± 10	34 ± 23	
dry cold	0.13 ± 0.01	53 ± 2.1	63 ± 4.1	
dry blanks	0.11 ± 0.03	34 ± 6.6	46 ± 13	
dry blanks batch 1	0.11 ± 0.04	31 ± 10	43 ± 23	
dry blanks batch 2	0.11 ± 0.01	39 ± 4.4	51 ± 8.0	

vents the liquid from freezing at $-20\text{ }^{\circ}\text{C}$, it is not known if *Natronorubrum* strain HG-1 employs additional mechanisms to prevent the formation of ice crystals at lower temperatures. These results suggest that *Natronorubrum* strain HG-1 could have survived cold periods that might have existed in the Earth’s history, e.g. 0.7–0.5 Gyr ago (Evans et al. 1997), but only if the salt concentration of the surrounding environment was high enough to keep the water liquid.

Desiccation

The desiccated blanks (figure 6.3c, DB) showed a slightly increased maximum growth rate ($\mu_{\max}$) and lag (L) compared to the wet (WB and WC) samples. Also, the inflection point (t_i) occurs later than that of the wet samples, which means that not all change in lag can be attributed to an altered growth rate, but an additional growth retarding mechanism must be in effect, depicted by

Table 6.3

Summary of the results. The results in this table are relative to the control experiment. The relation between the lag, the maximum specific growth rate ($\mu_{\max}$), and the inflection point (t_i) is given by equation 6.3. The ‘dry blanks’ sample is the average of batch 1 and 2.

experiment	lag (%)	$\mu_{\max}$ (%)	t_i (%)
control	100	100	100
wet blanks	69 ± 50	51 ± 17	95 ± 58
wet cold	69 ± 34	55 ± 20	94 ± 63
dry cold	178 ± 10	77 ± 9	172 ± 15
dry blanks	117 ± 22	67 ± 16	128 ± 38
dry blank b. 1	103 ± 35	66 ± 26	119 ± 63
dry blank b. 2	132 ± 16	69 ± 10	139 ± 24

arrow 2 in figure 6.2. Comparing the results for the samples that were in a desiccated state for 24 h (batch 1) with those desiccated for 48 h (batch 2), shows that the lag is increased when the cells remain longer in a desiccated state. These results show that desiccation has a small effect on the cell growth after rehydration, and that the effect is enhanced by prolonged desiccation. The dried samples that were analyzed with the Most Probable Number (MPN) method, have the same viable cell concentration after rehydration as the liquid control sample (table 6.4). This means that from the MPN analysis no change in viability due to desiccation could be detected. In the MPN analysis the first bottle that does not show growth is diluted by a factor 2 compared to the last bottle that displayed growth. This means

Table 6.4

Cell concentration and survival of the samples analyzed with the MPN method. The cell concentration is given by the highest dilution that still showed growth. The experiments that included Atacama desert soil did not show any growth, even for the undiluted culture.

sample	concentration (cells mL ⁻¹)	survival (%)
control	3×10^5	100
dried	3×10^5	100
dried on soil	0	0
dried & UV	10^2	$< 10^{-4}$
dried on soil & UV	0	0

that the relatively small change in lag between the wet blanks and the desiccated blanks in the growth curves experiment could be missed by the factor 2 step with the MPN analysis.

Adamski et al. (2006) have shown that cells of the bacterium *Pseudomonas aeruginosa* are incorporated in fluid inclusions, which are pockets of saturated brine in the halite crystals, and that these bacteria show motility while incorporated in those inclusions. It is likely that a large fraction of the *Natronorubrum* strain HG-1 cells will have been incorporated in inclusions in the salt crystals, similar to *Pseudomonas aeruginosa*. Cells that are incorporated in inclusions are not actually dehydrated, while the cells that remain outside the inclusions are more susceptible to dehydration and might not survive, caus-

ing increased lag in the growth curve. The lag increased further when samples were stored at -20°C for 24 h (figure 6.3b, DC samples). Those samples have a lag almost double that of the wet samples (WB, WC and control).

Mancinelli et al. (2004) found that *Halobacterium salinarum* NRC-1 and *Haloarcula*-G could survive repeated freeze-thaw cycles to -20 and -80°C for 144 days. The cells in those experiments were in the desiccated state. Mancinelli et al. (2004) furthermore found that control samples of *Escherichia coli*, *Pseudomonas fluorescens*, and *Deinococcus radiodurans* could not survive desiccation for 144 days at room temperature, at -20°C , or at -80°C . A notable exception was *D. radiodurans*, which survived freeze-thaw cycles to -80°C in the desiccated state for 144 days. Of the five bacterial strains tested, *H. salinarum* and *Haloarcula*-G were extreme halophiles which grow at 25 % NaCl. These findings concur with our results that halophilic organisms like *Natronorubrum* strain HG-1 can survive storage at low temperatures in a desiccated state.

Atacama soil

Natronorubrum strain HG-1 cultures were mixed with Atacama soil and subsequently dried. After rehydration, the MPN analysis showed no growth at any dilution (table 6.4). When *Natronorubrum* strain HG-1 cells were desiccated without soil, there was no measurable loss of viability. As we have established in the previous section, the cells are likely to survive in brine inclusions in the NaCl crystals (see also Mancinelli

et al. 2004). In a hypersaturated brine solution a nucleation point is needed for crystallization to start. In the experiments without soil, the cells acted as nucleation points and crystallization occurred around the cells. The NaCl crystal that formed around the cell, trapped water in an inclusion and prevented the cell from drying out. In contrast, when the cells were mixed with soil, the grains provided far better nucleation points than the cells. It is therefore likely that the cells in this mixture are left without a protective shell and dry out upon evaporation of the medium.

UV radiation

Dried samples of *Natronorubrum* strain HG-1 were irradiated for 30 min or 24 h. Figure 6.3c (DX & DD) shows that 24 h irradiation with either the solar simulator or the deuterium lamp completely incapacitated *Natronorubrum* strain HG-1. No growth was apparent after 120 h of incubation. *Natronorubrum* strain HG-1 samples that were irradiated for only 30 min, had a viable cell concentration of 10^2 cells mL^{-1} as analyzed by the MPN method (table 6.4), which is equivalent to a surviving fraction of 10^{-4} %. The *Natronorubrum* strain HG-1 samples that were mixed with Atacama soil, dried and subsequently irradiated, showed no growth at any dilution. This is likely due to the crystallization process that occurs in the soil, described in the previous section.

In general, cell death through interaction with UV radiation is associated with irreparable levels of DNA damage. This means that the pigments present in *Natronorubrum* strain HG-1

are not protective against light in the >200 nm wavelength range (see also figure 6.1). Additionally, OH radicals might be created by the UV photons from water present in the inclusions in which the cells are thought to reside (Adamski et al. 2006). Together with the fact that NaCl crystals are transparent for UV light down to ~ 200 nm (Li 1979), the formation of OH radicals that may be damaging to *Natronorubrum* strain HG-1 in fluid inclusions, is possible.

Combined effects

Desiccation of *Natronorubrum* strain HG-1 had a small effect on the viability (section 6.3), which increased when the desiccation period increased from 24 h to 48 h. From this short period of incubation in desiccated state, it is difficult to extrapolate the survival of *Natronorubrum* strain HG-1 over geological relevant time scales, but other halophilic archaea have been shown to survive for millions of years in inclusions in halite crystals (Stan-Lotter et al. 1999). Desiccated *Natronorubrum* strain HG-1 cultures did not survive irradiation with a solar simulator for 24 h (section 6.3). The solar simulator gave an output similar to the solar spectrum on Mars (see figure 6.1). *Natronorubrum* strain HG-1 would therefore not be able to survive on the surface of Mars for more than a few sols (martian days), unless they are protected from UV radiation, e.g. buried in the martian soil. Even when protected against UV radiation in the martian soil, survival of *Natronorubrum* strain HG-1 under martian conditions is doubtful, since the viability of desiccated cells was decreased by low (-20 °C) temperature (sec-

tion 6.3). Although the temperature on the surface of Mars can be above 0 °C near the equator at noontime, the average temperature is around -60 °C. Overall we conclude that *Natronorubrum* strain HG-1 can not survive the environment currently present on Mars.

6.4 Conclusion

We investigated the survival of the halophilic archaeon *Natronorubrum* strain HG-1 exposed to high temperature (70 °C), low temperature (4 and -20 °C), desiccation and irradiation with wavelengths >200 nm. Growth of *Natronorubrum* strain HG-1 was not affected by a 24 h storage at 4 or -20 °C. Desiccation of cells for 24 h or 48 h under ambient conditions had a negligible effect on the growth curves. When desiccated cells were stored at -20 °C for 24 h, there was an increase in the lag of the rehydrated culture. Cell cultures stored at 70 °C or desiccated in the presence of Atacama soil did not show any viability. Desiccated samples irradiated with UV light for 30 min showed a survival of $<10^{-4}$ %, while irradiation for 24 h completely sterilized the sample.

From these results we conclude that *Natronorubrum* strain HG-1 would not be able to survive on the surface of Mars, when unprotected from UV radiation. Even when protected from UV photons, viability would be impaired by a combination of desiccation and low temperature. *Natronorubrum* strain HG-1 could probably not have survived in the early Earth's oceans, due to its sensitivity to high temperatures.

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Summary

Many organic molecules have been detected in interstellar environments and even more are found in the solar system, for example in comets and meteorites. Some of these molecules are also found in living organisms on Earth, suggesting that there might be a connection between stardust and the origin of life on Earth. Before life could emerge on Earth, the building blocks for life had to be present. It is likely that both indigenous and external sources have contributed to the organic inventory on the early Earth.

In chapter 2, 3, and 4 of this thesis, the photostability of several organic compounds was analysed in simulated interstellar environments. The results show that nucleobases and their precursors are rapidly destroyed in most interstellar environments and in the solar system, if they

reside in the gas phase. These compounds are relatively stable in dark interstellar clouds, that experience a low UV radiation field. Small N-heterocyclic molecules may be formed in the circumstellar envelopes of carbon-rich stars, but have not yet been detected by astronomical observations. N-heterocycles with multiple nitrogen atoms in the ring are unlikely to be formed and are increasingly unstable against UV radiation. In combination with theoretical calculations, our results show that it is not likely that nucleobases and their precursors are present in significant abundances in interstellar and circumstellar regions.

Stars form by gravitational collapse of the clumpy cores of dense interstellar clouds. After formation of the protostar, the remaining dust

and gas accretes to form larger bodies and eventually planets. Organic material that was present in the presolar nebula is lost during terrestrial planet formation, because of the heat generated by the planet formation process. But organics can survive in the outer solar system and may be incorporated in small solar system bodies. These objects can hit the young planets, thereby delivering their organic content. Meteorites, fragments of comets and asteroids that reach the surface of the Earth, have been analyzed and found to contain a large variety of organic molecules, including amino acids and small amounts of nucleobases. Our results support the hypothesis that those molecules are formed in the presence of water on the parent bodies of meteorites, where they are protected from UV radiation.

Mars is annually supplied with 2.4×10^5 kg of carbon by meteorite impact. Despite this supply, the Viking landers did not detect any organic molecules in the martian surface above a detection limit of a few ppb. The discrepancy is usually attributed to oxidizing reactions on the surface and in the subsurface soil. In chapter 5 of this thesis the stability of amino acids in Mars soil analogues is investigated. The results show that the stability is dependent on soil mineralogy. While destruction of amino acids in Atacama desert soil and Orgueil meteorite samples is rapid, deposits from the Salten Skov region show negligible loss of amino acids after exposure to a simulated Mars environment. We conclude that

clay-type minerals can protect organic molecules against destruction. Our results provide support for future life detection missions to Mars and a proper landing site selection.

Data returned from recent missions to Mars, show that Mars probably had liquid water on its surface in the past. If organic compounds and a suitable energy source were available, life may have emerged on Mars. At a later stage, when water disappeared from Mars, the progressive desiccation led to an increase in salt concentration in the remaining bodies of water. Potential martian organisms would have to adapt to these high-salt conditions in order to survive. On Earth, viable halophilic microorganisms have been found in inclusions in 290–206 Myr old salt crystals. We have tested the survival of halophilic archaeon *Natronorubrum* sp. strain HG-1 under conditions similar to those found on Mars. The results in chapter 6 show that *Natronorubrum* strain HG-1 can be desiccated and rehydrated with a negligible effect on its growth, but that these organisms did not survive exposure to martian UV. *Natronorubrum* cells were also incapacitated by high temperature (70 °C), while low temperature (−20 °C) severely affected the growth. Cells did not survive desiccation in combination with Atacama desert soil. From these results we conclude that *Natronorubrum* strain HG-1 is not likely to survive in a martian environment.

Samenvatting

Als je op een heldere avond omhoog kijkt, zie je duizenden sterren aan de hemel staan. Naar schatting zijn het er zo'n 10^{22} , dat is 10.000.000.000.000.000.000.000. Sterren zijn geclusterd in sterrenstelsels. Ons eigen sterrenstelsel, de Melkweg, telt zo'n 230 miljard sterren. Lange tijd dacht men dat de ruimte tussen de sterren leeg was. De ruimte is inderdaad relatief leeg als je het vergelijkt met bijvoorbeeld de atmosfeer op aarde, maar helemaal vacuüm is zij niet. De ruimte tussen de sterren bevat een kleine hoeveelheid materie. Omdat er atomen in de interstellaire ruimte zijn, kan er chemie plaatsvinden en kunnen er moleculen gevormd worden.

Organische moleculen bestaan uit koolstof- en waterstofatomen, maar kunnen ook andere atomen bevatten, zoals zuurstof en stikstof. Al-

le levende organismen op aarde, van de simpelste bacterie tot complexe zoogdieren inclusief de mens, zijn opgebouwd uit een verzameling van kleine organische moleculen (zoals aminozuren, nucleotiden, suikers en vetzuren), grotere polymeren (bijvoorbeeld eiwitten, RNA en DNA) en zeer grote supramoleculaire complexen, waaronder ribosomen en membranen. De kleine organische moleculen kunnen dus beschouwd worden als de bouwstenen waarop het leven gebaseerd is. Deze bouwstenen moeten op een planeet aanwezig zijn, voordat er leven kan ontstaan. Mijn proefschrift gaat over de chemie van de bouwstenen van het leven, die plaats vindt tussen de sterren en op planeten.

Het ontstaan van sterren

Toen het heelal nog erg jong was, zo'n 100–300 seconden na de oerknal, werd alle materie in het heelal gevormd. Die materie bestond eerste instantie uit ongeveer 75% waterstof, 25% helium en minieme hoeveelheden deuterium, tritium, lithium en beryllium. Alle andere atomen zijn hier later uit ontstaan. Deze gassen koelden langzaam af en vormden grote wolken in het heelal. Na geruime tijd (de beste schatting is momenteel 300–500 miljoen jaar na de oerknal) ontstonden de eerste sterren uit die wolken gas.

Sterren ontstaan als wolken gas en stof door hun eigen zwaartekracht ineen storten. Daardoor ontstaat er lokaal een gebied met een hogere dichtheid, waardoor er nog meer gas wordt aangetrokken. In het binnenste zal door de toegenomen druk de temperatuur gaan stijgen. Uiteindelijk worden de temperatuur en druk zo hoog en wordt het gas in het binnenste zo dicht op elkaar gedrukt, dat de atoomkernen samensmelten en er kernfusie plaatsvindt. De ster is dan geboren. In de eerste fase van de ster wordt er door kernfusie helium gevormd uit waterstof bij een temperatuur van ongeveer 10 miljoen Kelvin. Nadat alle waterstof in de kern is omgezet naar helium, zal de kern van de ster weer verder samentrekken. Daardoor nemen druk en temperatuur weer toe. Bij een temperatuur van 100 miljoen K fuseren 3 heliumkernen tot koolstof. In een vervolgreactie wordt zuurstof gevormd uit koolstof en helium. Nadat alle helium in de kern van de ster is gefuseerd tot koolstof en zuurstof, kan de ster, mits zwaar genoeg, weer verder samentrekken en de volgende fase van kernfusie ingaan. De zwaarste

sterren gaan door met kernfusie tot er ijzer gevormd is. Daarna levert de kernfusiereactie geen energie meer op en stopt de keten. De allermassieve sterren kunnen dan nog verder samentrekken waarbij alle materie in de kern in één keer in neutronen wordt omgezet. Hierbij komt in korte tijd een enorme hoeveelheid energie vrij, een supernova explosie. Het resultaat van een supernova explosie is een neutronenster of een zwart gat.

Sterren die zich in de fase bevinden waarin ze koolstof en zuurstof produceren, worden *Asymptotic Giant Branch* (AGB) sterren genoemd. Stellaire winden blazen het geproduceerde koolstof en zuurstof weg van de ster. Als het gas voldoende afgekoeld is, reageren ze tot koolstofmonoxide (CO). Deze reactie gaat door tot één van de twee elementen verbruikt is. Als er meer koolstof dan zuurstof in de ster geproduceerd wordt, zal zuurstof als eerste op zijn en kan koolstof verder reageren. Dan worden moleculen zoals acetyleen (C_2H_2) en waterstofcyanide (HCN) gevormd. Een belangrijke reactie die kan plaatsvinden, is de polymerisatie van acetyleen tot benzeen (C_6H_6) en verder tot polycyclische aromatische koolwaterstoffen (PAKs). De stellaire winden blazen de gevormde moleculen verder naar buiten, waardoor het gas afkoelt. Als de temperatuur laag genoeg is, kunnen PAKs en andere moleculen samenklitten. Hierbij worden grotere, amorf stoffdeeltjes gevormd. Deze stoffdeeltjes houden het licht van de ster tegen, waardoor de temperatuur verder daalt. Ook het ultraviolet (UV) licht wordt tegengehouden, wat schadelijk is voor de meeste moleculen. Daardoor ontstaat er rond de ster een schil van stof die koud en donker is, waar moleculen beschermd

worden. Nog verder weg van de ster wordt het stof weer verdund en krijgt het interstellaire UV licht de overhand. Alleen de meest stabiele moleculen, de PAKs en de stofdeeltjes, zijn bestand tegen dat UV licht. Zij worden de vrije ruimte ingeblazen. Aan het eind van zijn bestaan heeft de ster een grote hoeveelheid stof en gas de ruimte ingeblazen. Dit stof en gas verrijkt het interstellaire medium, de ruimte tussen de sterren, waar het in grote wolken samenkomt. Uit deze wolken kunnen dan later weer nieuwe sterren ontstaan.

Interstellaire wolken

Het interstellaire medium kan in een aantal verschillende gebieden worden ingedeeld. Het neutrale interstellaire medium heeft een temperatuur van ongeveer 10.000 K en een dichtheid van 0,1 deeltje per cm^3 . Diffuse wolken hebben een temperatuur van ongeveer 100 K en een dichtheid van 10-100 deeltjes per cm^3 . Door deze lage dichtheid heeft het interstellaire UV licht vrij spel. Donkere wolken hebben een veel hogere dichtheid (1000–100.000 deeltjes per cm^3), waardoor er geen licht tot het binnenste van de wolk door kan dringen. Daardoor is de temperatuur ook laag, <50 K. Dit heeft tot gevolg dat in donkere wolken de meeste moleculen niet gasvormig zijn, maar als ijslaagjes op de stofdeeltjes vastgevroren zitten. Hierdoor kan er een heel ander soort chemie plaatsvinden dan in de gasfase. Een groot aantal moleculen zijn al gedetecteerd in donkere wolken, zoals water (H_2O), koolstofmonoxide (CO), koolstofdioxide (CO_2), methanol (CH_3OH) en ammoniak (NH_3).

Zoals eerder al beschreven, ontstaan sterren

uit wolken gas en stof. Dat begint met het vormen van regio's met een verhoogde dichtheid. Deze regio's worden *hot (molecular) cores* genoemd, of *hot corinos* als het om protostellaire objecten met een lage massa gaat. Hot cores hebben een temperatuur van 100–300 K en een dichtheid groter dan 1 miljoen deeltjes per cm^3 . Omdat de temperatuur is gestegen, verdampen de ijslaagjes die zich op de stofdeeltjes afgezet hebben. De moleculen die hierbij vrij komen, verrijken de gasfase en leiden weer tot nieuwe chemische reacties.

Planeten

Sterren vormen uit samentrekkende wolken gas en stof. Maar niet alle stof en gas uit de wolk eindigt als brandstof voor de ster. Een klein deel van de stofwolk blijft achter. Die stofwolk begint rond te draaien en wordt daardoor vervormt tot een stofschiif. In deze schijf botsen de stofdeeltjes tegen elkaar en blijven aan elkaar plakken. Hieruit ontstaan steeds grotere objecten. Als deze objecten een doorsnede van een kilometer hebben, gaat aantrekkingskracht een rol spelen en zal de accretie sneller verlopen. Objecten van een kilometer of meer worden planetisimalen genoemd. Als de objecten zo groot zijn dat zij door zelfgravitatie een ronde vorm krijgen, dan worden het protoplaneten genoemd. Deze planeten hebben een doorsnede van minimaal ongeveer 1000 km. Wanneer de protoplaneten alle materiaal in hun baan hebben opgeslokt of weggeslingerd, dan zijn het volwaardige planeten geworden.

Niet alle brokstukken worden door de planeten opgeslokt. In ons zonnestelsel bevindt zich

tussen Mars en Jupiter een baan, de asteroïdengordel, met overgebleven rotsblokken die niet in de planeten terecht zijn gekomen. Deze asteroïden zijn waarschijnlijk de oorzaak van meteorieten die we op aarde vinden. Door botsingen in de asteroïdengordel kunnen brokstukken uit hun baan geslingerd worden, richting de aarde. Uit analyses blijkt dat er op sommige meteorieten een groot aantal verschillende organische moleculen voorkomt, waaronder aminozuren. Metingen aan de isotopenverhoudingen in die aminozuren hebben aangetoond dat de aminozuren geen vervuiling van levende organismen op aarde zijn, maar werkelijk uit de ruimte komen.

Voorbij de planeet Neptunus zijn nog twee reservoirs van brokstukken. Omdat deze brokstukken zich ver van de zon bevinden, zijn ze omgeven door een dikke laag ijs. Deze ijzige brokstukken zijn kometen en zweven rond in de Kuipergordel en de Oortwolk, genoemd naar de twee Nederlandse ontdekkers Gerard Kuiper en Jan Oort. Net als asteroïden en meteorieten kunnen kometen inslaan op planeten. Daarbij kunnen ze hun inhoud aan organische moleculen op de planeet afleveren. Als op die planeet een vloeibaar medium voorhanden is, bij voorkeur vloeibaar water, kunnen chemische reacties op de planeet de complexiteit van de moleculen vergroten. Deze chemische ‘evolutie’ kan leiden tot complexe structuren met emergente eigenschappen, waaruit uiteindelijk leven kan ontstaan.

Mars

Mars is de planeet in ons zonnestelsel die het meeste op de aarde lijkt. De Vikingmissie uit

1976 heeft gezocht naar de aanwezigheid van leven en organisch materiaal op Mars, maar niets gevonden. Later zijn er enkele bezwaren gerezen over de kwaliteit van de resultaten, maar desondanks is het verbazingwekkend dat er geen enkel organisch molecuul is gevonden. Er valt jaarlijks namelijk ongeveer 240.000 kg koolstof neer op Mars via meteorietinslagen en interplanetaire stofdeeltjes. Toch hebben de Vikinglanders dit niet gevonden. Het huidige model gaat er vanuit dat de organische stoffen door oxidatiereacties op het oppervlak en in de bodem van Mars afgebroken worden.

In de afgelopen tien jaar zijn een aantal succesvolle satellieten en landers naar Mars gestuurd, die foto's van het oppervlak hebben gemaakt. Op die foto's zijn geulen te zien die vergelijkbaar zijn met riviergeulen op aarde. Daarnaast zijn er verschillende soorten mineralen gevonden, zoals jarosiet en hematiet, die normaal in de aanwezigheid van water gevormd worden. Hieruit heeft men afgeleid dat er in het verleden water op Mars moet zijn geweest gedurende een geologisch relevante periode. Ook nu is er nog water op Mars, al zit dat opgeslagen in ondergrondse ijslagen.

Het proefschrift

In het bovenstaande stuk heb ik een korte impressie gegeven van het ontstaan van sterren en planeten, de chemische omstandigheden in het heelal en op planeten, specifiek op Mars, en de manier waarop buitenaardse chemie kan bijdragen aan het ontstaan van leven. Voordat een organisch molecuul uit de ruimte kan bijdragen

aan het ontstaan van leven, zal het eerst intact op een planeet moeten aankomen. Voor het onderzoek beschreven in dit proefschrift heb ik gekeken naar de stabiliteit van organische moleculen onder gesimuleerde ruimte- en planetaire omstandigheden. Daarnaast heb ik de overleving van een levend organisme op Mars bestudeerd.

Dimethylether is een van de grootste organische moleculen die in de ruimte is gedetecteerd. Het is gevonden in de gasfase van hot molecular cores. In **hoofdstuk 2** wordt de afbraaksnelheid van dimethylether door UV licht beschreven, gemeten onder gesimuleerde ruimteomstandigheden in het laboratorium. De resultaten werden gebruikt om chemische modellen van een hot molecular core aan te scherpen. Dimethylether bleek snel af te breken onder invloed van UV licht, maar dat bleek slechts van kleine invloed op de chemische modellen van hot molecular cores.

Stikstofhoudende cyclische organische moleculen (N-heterocycles) zijn belangrijk voor het leven op aarde. Zo zijn de nucleobasen uit DNA gebaseerd op N-heterocycles. In **hoofdstuk 3** worden de afbraaksnelheden van pyridine, pyrimidine en s-triazine beschreven, gemeten onder gesimuleerde ruimteomstandigheden. Uit de resultaten blijkt dat de afbraaksnelheid toeneemt, als het aantal stikstofatomen in de ring groter wordt. Vertaald naar de interstellaire ruimte betekent dit dat alle drie de moleculen snel afgebroken worden in het diffuse interstellaire medium, maar dat pyridine en pyrimidine stabiel zijn in donkere wolken gedurende de gemiddelde levensduur van die wolk. Verder wordt een mogelijke vorming van pyridine in de gasfase gegeven

en wordt er beredeneerd dat het niet waarschijnlijk is dat er meer dan één stikstof per ring ingebouwd zal worden.

Nucleobasen zijn belangrijke onderdelen van DNA en RNA en daarmee de dragers van genetische informatie in alle levende organismen op aarde. In meteorieten zijn nucleobasen gedetecteerd, die mogelijk een buitenaardse oorsprong hebben. Er zijn echter nog geen isotoopverhoudingen gemeten om een buitenaardse oorsprong definitief aan te kunnen tonen. **Hoofdstuk 4** beschrijft de UV fotostabiliteit van de nucleobasen adenine en uracil. Beide worden snel afgebroken worden in het diffuse interstellaire medium en in het zonnestelsel ter hoogte van de aarde, maar deze moleculen stabiel zijn in donkere wolken, minstens zo lang als de gemiddelde levensduur van zo'n wolk. Hieruit wordt geconcludeerd dat deze moleculen moeilijk te vormen zijn in de gasfase en dat ze in het zonnestelsel waarschijnlijk gevormd worden op de asteroiden of kometen waar meteorieten vandaan komen.

Op Mars komt jaarlijks naar schatting zo'n 240.000 kg koolstof terecht door inslagen van meteorieten en stofdeeltjes. De Vikingmissie heeft echter geen enkel organisch molecuul kunnen vinden. Deze discrepantie wordt meestal toegeschreven aan oxidatiereacties in de bodem van Mars. In **hoofdstuk 5** laten we de stabiliteit van aminozuren in verschillende bodemsoorten op aarde zien, die gebruikt worden als analoga van de Marsbodem. In de experimenten wordt zand uit de Atacama woestijn van Chili en Peru vergeleken met een ijzerrijke bodem uit Denemarken en een gemalen stukje van de Orgueil meteoriet. De afbraaksnelheid van de aminozuren blijkt sterk

af te hangen van de mineralogie van de bodemsoort. Kleiachtige grondsoorten blijken in staat om de aminozuren tegen afbraak te beschermen.

Halofiele archaea zijn micro-organismen die groeien bij hoge tot verzadigde zoutconcentraties. In **hoofdstuk 6** wordt het halofiele archaeon *Natronorubrum* sp. stam HG-1 blootgesteld aan hoge (70 °C) en lage (4 en –20 °C) temperaturen, aan UV licht, aan uitdroging en aan zand uit de Atacama woestijn. Uit de resultaten blijkt dat lage temperatuur geen invloed op de groei van *Natronorubrum* strain HG-1 heeft, maar dat na incubatie bij 70 °C er geen enkele groei meer plaatsvindt. Uitgedroogde culturen kunnen volledig herstellen, maar als de uitdroging gecombineerd wordt met incubatie bij –20 °C, is er verminderde groei. Zowel UV licht als mengen met Atacama grond zorgt voor volledige sterilisatie van *Natronorubrum* strain HG-1. Hieruit wordt geconcludeerd dat *Natronorubrum*

strain HG-1 weinig kans heeft om op Mars te kunnen overleven.

Uit het onderzoek beschreven in dit proefschrift, blijkt dat de bouwstenen van het leven op aarde slecht bestand zijn tegen de omstandigheden in de ruimte. Door het sterke UV licht dat op veel plaatsen in de ruimte aanwezig is, worden deze organische moleculen snel afgebroken. Ook lijkt de vorming van deze moleculen moeilijk te zijn in veel gebieden in de ruimte. Binnen ons zonnestelsel worden deze bouwstenen waarschijnlijk gevormd op de asteroïden en kometen waar meteorieten vandaan komen, aangezien een aantal van de bouwstenen van leven op meteorieten zijn gevonden. Ook op een planeet zoals Mars, zijn de bouwstenen aan afbraakprocessen onderhevig. De bouwstenen zullen dus goed beschermd moeten worden voor ze bij kunnen dragen aan het ontstaan van leven.

Acknowledgement

Disclaimer — this acknowledgement is entirely based on my memory and, being human, my memory is fickle. I have come to accept that fact and I hope you do to. If you are inadvertently unmentioned here, do not feel yourself unacknowledged. Please, mention it to me and I will thank you personally!

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Het is klaar.

Curriculum Vitae

5 April 1977 was the joyful day that I was born. I enjoyed my early education at the basisschool st.-Pieter(1983–1989) in Ommel and st.-Willibrord gymnasium in Deurne (1989–1996). In 1996, I went on to study chemistry at the Katholieke Universiteit Nijmegen (now Radboud Universiteit Nijmegen). I did a major in biochemistry under the supervision of prof. Wim de Grip on the subject of eye-pigment proteins, the rhodopsins and the cone-pigment proteins in 1999–2000. One of the optional courses I took in my undergraduate years was the course ‘Introduction to Astrophysics’ by the highly inspiring prof. Jan Kuipers. He ignited my interest in astronomy and suggested that I should have a look in Leiden where prof. Pascale Ehrenfreund was combining astronomy with chemistry and biology. From 2001–2002, I did an undergraduate project with Pascale Ehrenfreund at the Leiden University. After receiving my Master’s degree in chemistry from the Katholieke Universiteit Nijmegen in 2002, I returned to Leiden at the start of 2003 to begin my PhD career under the supervision of Pascale Ehrenfreund, the result of which lies before you now. During the past 5 years I have presented my research at conferences in Baltimore (2002), Madrid (2003), Reykjavík (2004), Budapest (2005), Hawai’i (2005), st. Jacut de la Mer (2006), and Vienna (2007). After defending my PhD thesis I hope to continue as a post-doctoral researcher in the broad field of Astrobiology.

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