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REVIEW

Fullerenes toxicity and electronic properties

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Abstract Nanotechnology globally represents a new direction within scientific development, where the atomic and electronic properties of molecules are used in a unique fashion to produce and construct new and exotic materials and products. Fullerenes (Bucky balls, C_{60}) constitute a particular group within the field of nanotechnology. Fullerenes find applications in medicine, industrial chemistry and electronics. However, there are several unanswered questions about fullerenes and their toxicological properties. Most toxicological studies on fullerenes evolve around the in vitro and in vivo aspects of pristine C_{60} along with chemically modified C_{60} molecules. We reviewed toxicology reports on C_{60}. We bring a critical and challenging evaluation of the electronic and quantum properties of C_{60} molecules in context with the implications on cellular factors and metabolites. The evaluation shows that the reactivity and quantum chemical properties of C_{60} can have unexpected effects in the cell, by principally absorbing metabolites, such as OH^- and H^+ ions and alter its reactivity. We thus challenge the present view of C_{60} solely based on empirical studies, based on the electronic properties of C_{60} that vary considerably with their size and reaction path. A further example of this is the absorption of divalent zinc ions, which shows an increase in reactivity of the C_{60} that presents an important pattern of chemical state, reactivity and toxicological potential. The results evaluate the toxicological potential of C_{60} from a different angle than conventional, by applying a blend of critical review of the findings on C_{60} toxicity, their chemical and electronic properties.

Keywords Fullerenes · Chemical modifications · Toxicity prediction · Electronic properties · Quantum chemistry · Mutagenicity

Introduction

Nanotechnology is a novel arena of science which is gradually becoming a large producer of products, some of which may have toxic properties (Tanaka 2004; Miles et al. 2005). The environmental fate of nanoproducts and potential health-adverse effects is gradually being studied, but unfortunately *after* the implementation of many new products (Elliot 2011). The production of nanomaterials encompasses carbon nanotubes, fullerenes, graphene sheets and many other formats of carbon nanomaterials for the improvement of technological, industrial and medical products.

Fullerenes, such as Bucky balls, C_{60}, are a group of carbon nanomaterials formed as spherical nanoensembles which range from C_{20} to C_{720} (and larger), typically forming empty closed cage structures, which are made up of multiple 5-member and 6-member rings (Fig. 1). They are applied primarily in medical and bio-nanotechnology

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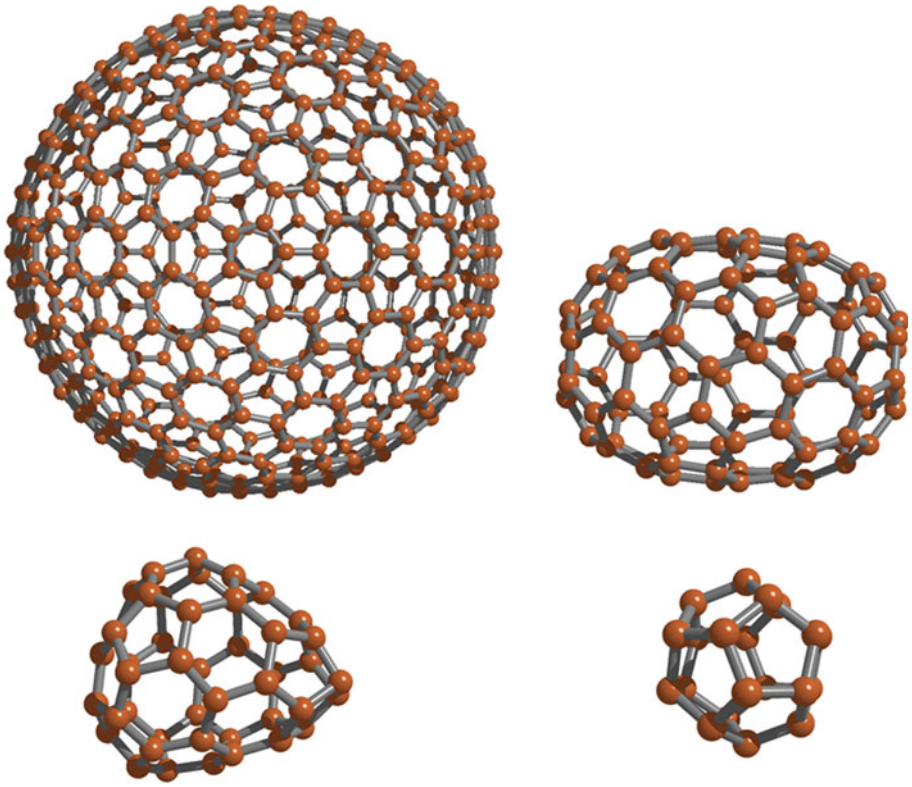

Fig. 1 Various fullerenes in different shapes and sizes. From *top left* to *bottom right*: a C500 fullerene; a C96 fullerene; a C46 fullerene; a C20 fullerene. The 6-carbon rings are seen adjacent to the 5-carbon ring in a well-organized manner, and the smaller they become, the more 5-member rings they gain, increasing also their reactivity. Image generated in Argus Lab (Thompson 2004), models generated in Nanotube Modeler (JCrystalSoft 2012)

structures, given that they exhibit a wide range of biologic activities (Zhou et al. 2010). Areas of applications include transport of drugs through dense tissues, for instance in tumors, DNA photo-cleavage, and gene delivery (Da Ros 2008). Fullerenes are also applied in environmental remediation, where they are used in pathogen decontamination (Mauter 2008). In energy systems such as in organic solar cells (Shrotriya et al. 2006; Yuan et al. 2011), the unique and highly versatile electronic properties of fullerenes are taken advantage of. An example of such properties is the case where upon illumination as part of a solar cell, fullerenes liberate electrons which are generated at the junction with the resin they are bound with (Anctil et al. 2011). In other words, fullerenes give completely novel aspects to these various applications in the industry.

An important property of fullerenes is the capacity to pass membranes (Jensen et al. 1996), and this allows use in drug delivery. An example of a direct application in medicine demonstrated the ability of fullerenes to down-regulate the oxidative stress in the lung tissue of tumor-bearing mice (Jiao et al. 2010). Yet other examples have been reported of selective drug delivery, such as in the context of diabetes (Hosseini et al. 2010). In medical screening, fullerenes are also applied, such as in the case where single metal atoms are encapsulated in a Bucky ball to serve as absorbers in biomarkers (Zhang et al. 2010). Furthermore, fullerenes have also been applied in combination with biomolecules, in order to promote specific functions, such as catalysis of hydrogen peroxide (Willner and Willner 2010).

The chemical reactivity of fullerene C_{60} has been known for several years, and it is a common agreement that there are several different chemical classes of C_{60} derivatives. Like benzene derivatives, for example, toluene, nitro-toluene or phenylalanine, each C_{60} derivative has its own physicochemical characteristics and toxicological behavior. In order to simplify the task of reporting toxicological properties of C_{60} and supplying valuable computational data, the authors focus on pristine C_{60} in particular.

The health-adverse aspects of C_{60} have been reported, debated and opposed since the early 1990s. The knowledge on their effects on living organisms is, however, increasing, and several important issues arise and are summarized in the following sections.

Health-adverse aspects of fullerenes

Fullerenes have been widely studied for health-adverse aspects in the last years and have shown to be interfering with several mechanisms in the body of animals and humans. These effects vary according to the format of the fullerenes, and their chemical modifications. However, modified, the principal toxicological property of fullerenes is due to their predominantly apolar character which is

compatible with merging with biologic membranes in organism (Nakagawa et al. 2011; Sadauskas et al. 2007).

In the study by Nakagawa et al. (2011), hydroxylated fullerenes, also named fullerenoles, were tested on rat hepatocytes. The fullerenes showed strong detrimental effects and induced cell death within the 3 h in the rat liver cells. Furthermore, several crucial cellular factors, such as ATP and thiol levels, were depleted by the fullerenes. The fullerenes were also found to cause substantial damage on the mitochondrial membrane, particularly the highly hydroxylated fullerenes types which induced chemical modification of the lipid molecules. It was found that out of the several membranes in hepatocytes, the mitochondrial membrane was affected most negatively. This led directly to dysfunction of the Adenosine-5′-triphosphate (ATP) synthesis and cell death. This study therefore introduces the important notion that the modification from pristine C_{60} to hydroxylated C_{60} makes the hydrophobic particle more hydrophilic while simultaneously introducing other side effects. This mechanism presents a unique and perhaps novel form of toxicity, where innocuous molecules such as water are transported by the toxic agent into domains within the cell where they become detrimental and act as co-contaminant. This mechanism could in principle work with other co-contaminants.

An additional observation is that modified fullerenes may become more bio-compatible if organic and or charged adducts are present. Due to the various modifications, fullerenes may resemble cellular components, particularly proteins, because of the spherical structure, amphiphilic nature and stable conformation. This may therefore make it is easier for fullerenes to pass "unhindered" by the body's defense system, making modified fullerenes harder to be identify and degrade, compared to non-hydroxylated fullerenes. Such a mechanism of increased toxicity through increasing bio-availability via "bio-resemblance" was observed by Drug et al. (2011), where toxins were more efficiently transported throughout the lungs due to their compatibility with the tissue.

Biocompatibility can thus also facilitate hormonal disturbances and other pathophysiological effects. The mapped studies are reported below in categories.

Hormonal interference of fullerenes

Hormonal influence from fullerenes is directly shown in animal studies, mainly on fish studies. In one study, in particular, the effects of pristine C_{60} were assessed toward the ability of zebrafish to uptake hormones (Park et al. 2010). The formed nanoaggregates reduced the uptake of estradiol derivates in fish, by adsorbing them. This induced hormonal changes in the zebrafish and showed that pristine C_{60} in aquatic environment gave indeed bio-interfering effects.

Pristine C_{60} are known to form aggregates in aqueous solution, of up to multiple nanometers in diameter and hundreds of nm in length (Park et al. 2010) and bind strongly to organic matter (Hyung et al. 2007; Li et al. 2009) and has the potential of depleting organic nutrients from aquatic habitats (Park et al. 2010). Also, pristine C_{60} are interestingly absorbed in the small intestine of fish, reaching almost 100 % of uptake in zebrafish (Ibid.), which allows the C_{60} in passing the intestinal blood barrier.

Observations on the nature of pristine C_{60} aggregates in aqua-toxicological studies have also been made by Jovanovic et al. (2011) and Kim et al. (2010) who used different carriers of pristine C_{60} in their study on fish embryos. Their results showed how aggregates of pristine C_{60} in combination with aromatic compounds (experimentally used and tested as carriers) inhibited neutrophil function and affected embryonic development. This implied that they characterized the studied fullerenes as teratogenic.

These results showed that the most occurring consequence of the intoxication of pristine C_{60} suspended in toluene in fish embryo was mortality, while the second was deformity. Pristine C_{60} was found in the study of Tsuchiya et al. (1996) to be able to carry/be carried through the embryonic cells by dimethyl sulfoxide (DMSO), a strongly polar compound. This combination caused a much higher effect on malformation and delayed development of the fish embryos. In combination with water only, pristine C_{60} induced mortality in 28 % of the embryos.

The explanation for these effects is the ability of pristine C_{60} to penetrate the cell membrane (Qiao et al. 2007), and its ability to bind co-contaminants. In a co-contaminated situation, as for instance in an oil/gas leakage, the toxicity of fullerenes can therefore increase, according to a study with different carrier molecules (Fortner et al. 2005). However, inversely, the function of pristine C_{60} as environmental remediators has also been mooted (Mauter 2008), which instigated a positive function of pristine C_{60} under controlled conditions.

As discussed above, the availability of pristine C_{60} suspensions in aquatic environments may affect reproduction, and create problems for marine species that rely on smaller organisms which attempt to metabolize or are intoxicated by pristine C_{60} aggregates. Since it is already known that pristine C_{60} aggregates are toxic to bacteria, their negative impact on ecosystems at large, from the simplest organism upward to more complex organisms, is known (Lyon et al. 2005; Fortner et al. 2005).

Because of the dependency of bacteria and monocellular organisms such as diatoms in the circulation and transformation of gases from the aqueous phase to the atmosphere, the presence of pristine C_{60} in modern society at high concentrations, for example, through consumer materials,

can therefore pose a significant risk, eco-toxicologically and toxicologically. This emphasizes the importance of studying fullerenes within aqueous environments in environmental sciences for the specific purpose of mapping trans-ecosystem consequences so as to document eventual long-termed effects between land, ocean and air ecology, if such products are to be incorporated into daily life as much as plastics have been in the last decades (Walsh 2010).

Tissue localization of fullerenes

In a study on the effects from fullerenes on largemouth bass, Oberdörster proved that pristine C_{60} induced oxidative stress in the brain of juvenile fish (Oberdörster 2004a). The action of oxidative stress was caused by the ability of pristine C_{60} to peroxidate membrane lipids and decrease glutathione levels. In context with findings by Nakagawa et al. (2011), the potential of a leakage of biochemical components from the brain cells in the juvenile fish is most likely the foundation for cell pathology by pristine C_{60}, as sustained by their ability to disrupt and disturb cell membrane permeability. The work by Oberdörster (2004a) showed that the brain cells were most susceptible for attack by the pristine C_{60}, and that they experienced a 17-fold increase in lipid peroxidation as compared to the negative control.

Opposite findings were revealed, however, for a nanoformulation of pristine C_{60} in a study by Tong et al. (2011) who showed that this particular configuration of pristine C_{60} was not toxic. The results shown here were, however, not on living organisms, but on neuronal cells, which may indicate a significant deficiency in the methodology for assessing bio-toxic effects of pristine C_{60}.

In this context, the pathway of uptake and degradation of pristine C_{60} in a multicellular organism is seemingly more complex than in a cell culture, and therefore, this study does not present unilateral confirmation on that this variant of pristine C_{60} is non-toxic as stated by Tong et al. (2011).

A study on rats by Yamago et al. (1995) showed that fullerenes remain in the body for weeks if absorbed by the system and when injected intravenously. In addition, it was found that the pristine C_{60} penetrates the blood–brain barrier and induces acute toxicity. Their results showed also, in concert with earlier findings (Nakagawa et al. 2011; Sadauskas et al. 2007) that fullerenes primarily deposit in the liver (Tong et al. 2011).

Pristine C_{60} and nanotoxicological properties

The dispersion of pristine C_{60} molecules in both air and aquatic sources may present a novel and special case of environmental and health-related hazards. This can be deduced from the observations discussed above, which indicate significant damage induced by pristine C_{60}, but also because the presence of particles with properties such as pristine C_{60} in air and aquatic sources may have more adverse effects than previously assumed. This is coherent with the observations by Oberdörster et al. (2005). Their review encompasses a vast amount of different nanoparticles as potential sources for toxicological and eco-toxicological effects. It is being pointed to that pristine C_{60} are crucial to study, due to the fact that a given *concentration* of fullerenes in a work environment or habitat, that would seem low compared with other pollutants, would represent a much higher effective "*molecule number*" than other conventional pollutants, because of the large number of atoms in the pristine C_{60} architecture.

Also, accounting for that most of these molecules are quite susceptible for modifications and interactions with other compounds in the environments, pristine C_{60} may be the source of *several* toxicological derivates. This notion changes the landscape of toxicological aspects of nanoparticles such as pristine C_{60}, because their action occurs primarily because of their special molecular quantum toxic properties and because they spread efficiently like dust particles or aerosols.

However, other studies (Henry et al. 2007, 2011; Mori et al. 2006; Sayes et al. 2007; Oberdörster 2004a, b) indicate that the results by Oberdörster et al. (2005) are not completely reflecting an exact nature of the toxicity of fullerenes. Several studies suggest instead that the toxic effect shown is a consequence of tetrahydrofuran used in the experiments. The adsorbing nature of pristine C_{60} of chemicals may thus be the only principal finding, and not the direct toxicity of fullerenes.

Because of these relationships between dose and particle number, the toxicological potential and multitude of chemical derivates that may arise from pristine C_{60} are vast and complex. The knowledge of the impact of pristine C_{60} on the body, including detrimental mechanisms, is far from complete to date. There is, however, some consensus on that the mechanism of fullerenes evolves around affecting the lipid-rich brain tissue after crossing the blood barrier and thereby induces oxy-radical production by microglia, or alternatively generates reactive pristine C_{60} (Oberdörster et al. 2005; Oberdörster 2004b). The "activated pristine C_{60} metabolite" is quite a probable scenario, similar to the case of biologically activated polycyclic aromatic hydrocarbons (PAHs) (Levin et al. 1982). This is due to their electronic properties, which we describe in the next chapter. The "activated metabolites" of pristine C_{60} can be produced through the cytochrome P450 metabolism and monooxygenase cascades to generate a number of inert or active metabolites as demonstrated on benzo[a]pyrene. However, this has to date not been demonstrated and could present a crucial avenue in nanotoxicological research.

Alternative theories of the destructive mechanisms of action of fullerenes are that pristine C_{60} direct redox-activity reaching the brain via circulation or axonal translocation (Oberdörster et al. 2005; Oberdörster 2004b). The process of de-toxification of pristine C_{60} may in fact result in increased toxicity, given that hydroxylation and solubilization of pristine C_{60} is the easiest route out of the body as conventionally known in phase I and phase II reactions (Dekant 2009). However, hydroxylated pristine C_{60} were shown to be more toxic than non-hydroxylated pristine C_{60} on rat hepatocytes (Nakagawa et al. 2011), and hence, pristine C_{60} may *increase* toxicity during inflammatory responses and detoxification pathways.

Pristine C_{60} and reactive oxygen species

Pristine C_{60} are known to induce the production of reactive oxygen species (ROS), which introduces the first inflammatory response of cell pathology, overloading the anti-oxidant defense system (Oberdörster et al. 2005; Cagle et al. 1999). This generates oxidative stress in the cell, and causes intra-cellular disturbances. In a study by Yamakoshi et al. (2003), the production of superoxide has been linked to pristine C_{60} as well. Generation of ROS species can interestingly lead to increased modification of pristine C_{60} molecules. ROS species and fullerenes can thus be "partners in crime" and create a codependent inflammatory reaction through ROS generation and pristine C_{60} active-metabolite production.

The duration of such ROS reactions is not known, however, the clearance of metal-ligated C_{60} from the liver in rats was observed to take about 5 days (Dekant 2009). Not answering directly the potential incubation time of metal-ligated variants of C_{60} can, however, be significantly easier to detoxify, given the polarizing effect by the metal ions.

Given the various types of modified and non-modified fullerenes with diverse chemical properties, it is emphasized that fullerenes as a general class of molecules may have widely different absorption rates and periods of duration within the body. Studies emphasizing chemical modifications and bio-responses in categorized manner may thus be beneficial to the scientific community in further understanding the debated effects of these exotic nanoparticles.

Mutagenic effects of pristine C_{60}

Fullerenes have been shown to interfere with DNA. For instance, pristine C_{60} fullerenes induced genotoxic effects on a human lymphocyte cell model, with strong correlation with the concentration of pristine C_{60} (Yamakoshi et al. 2003). The results also showed that quite low concentrations of pristine C_{60} were required to induce negative effects on the cell model (2.2 μg/L). Additionally, the size distribution of pristine C60 aggregates was observed to be highly variable, where both single molecules and multi-molecule aggregates of pristine C60 dispersed in the ethanol and aqueous solutions were found. The behavior of fullerene C_{60} in solution is therefore the foundation for generating nanoaggregates of these dimensions, which in turn are responsible for many of the effects observed (Nakagawa et al. 2011; Li et al. 2009; Henry et al. 2007; Yamakoshi et al. 2003).

Interestingly, given the electronic properties (which we discuss later in this review), larger fullerenes, such as pC_{70} may behave completely different in water, causing very different effects than pristine C_{60} and these molecules may potentially also target different tissues or cell types. The work on tissue culture and in vivo models may therefore only give a partial picture of the potential of fullerenes and their toxicological aspects, and in order to obtain a complete picture of the toxicity of a specific fullerene molecule, such tests are not sufficient.

The results from the study by Yamakoshi et al. (2003) showed also that pristine C_{60} aggregates produce oxygen radicals, as noted previously (Oberdörster et al. 2005; Yamakoshi et al. 2003; Markovic et al. 2007). Oxidated C_{60} (carboxy-C_{60}) has been shown to cleave DNA sequences at Guanine sites (Markovic et al. 2007; Dhawan et al. 2006; Subach et al. 2009; Tokuyama et al. 1993; Zhang et al. 2009). The mechanisms of DNA damage appear to depend on either reactions of C_{60} with reactive oxygen species in the nucleus promoting cleavage of DNA (Markovic et al. 2007), or through its photo-oxidated carbons interacting with water molecules (Dhawan et al. 2006). An alternative scenario is proposed here, where the photo-oxidated carbon atoms of fullerenes may potentially yield the reactive *carbanion* (carbon atom with two lone electrons), in a similar mechanism as observed in proteins by Subach et al. 2009), which becomes reactive with the DNA. A further possibility is elucidated through the photo-induced carboxylic activity of fullerenes (Tokuyama et al. 1993).

Nevertheless, pristine C_{60} has been found to pass through the cell membrane within a period of 15–30 min whereas the reactions occurring within the cell may hence commence shortly after intoxication (Zhang et al. 2009). However, a surprising toxicological aspect of pristine C_{60} is their ability in *avoiding* lysosomes (Zhang et al. 2009), which form the cells natural defense system against foreign matter in phagocytes. This feature stresses the unusual toxicological properties of pristine C_{60} that allow them to bypass the defense mechanisms of the cell. When lysosomes are unable to engulf the C_{60} and destruct them, the secondary reaction becomes by necessity the free expression of ROS within the cytoplasm. Freeing ROS imposes a risk since ROS species

can attack the cells own components as well. Based on the reactions of fullerenes in fish as referred to earlier (Park et al. 2010; Henry et al. 2007; Jovanović et al. 2011; Kim et al. 2010; Tsuchiya et al. 1996) and on the genotoxicity issues reviewed here (Markovic et al. 2007; Dhawan et al. 2006), we conclude that lysosome avoidance increases the problems for cells substantially and may participate as a central part of the toxicity mechanism of pristine C_{60}.

Pristine C_{60} distribution and degree of toxicity

The bio-distribution of pristine C_{60} in various organs is a question which is still poorly studied. So far, mostly fish species and rats have been assessed for localization of pristine C_{60} aggregates after intoxication. The results differ. In rats, the primarily deposition organ is the liver (Nakagawa et al. 2011; Sadauskas et al. 2007; Walsh 2010), while in the Japanese fish Medaka (*Oryzias latipes*), C_{60} depositions were found in the eggs, the gills, the intestines and the gallbladder (Kashiwada 2006). In largemouth bass, distribution of C_{60} in the brain has been studied (Oberdörster 2004a, 2004b). On the predator algae *Daphnia Magna*, the fullerenes were stuck in the filter-feed system where they inhibited absorption of nutrients (Oberdörster et al. 2006; Filella et al. 2008), while in juvenile carp, the liver resulted to be the main organ of deposition of pristine C60 fullerenes (Zhu et al. 2008).

The different rates of uptake and release, and the varying localizations of pristine C_{60} are particularly important to clarify. Some studies delineate that pristine C_{60} had no or low inflammatory effects on the lungs, from which it henceforth was concluded that pristine C_{60} as being innocuous (Fujita et al. 2009). Such studies therefore only give a limited image of the whole toxicological picture of pristine C_{60}, because of the different reaction of pristine C_{60} to different biochemical and chemical environments. For instance, the liver, which hosts a range of lipoproteins and apolipoproteins, cholesterol- and hormone-processing enzymes and substrates, contains a vast array of biochemical compounds, some of which have a high affinity for interaction with pristine C_{60}, due to their preference for hydrophobic substrates.

The lung, which in addition to a group of water-soluble inflammatory factors contains tissue-specific surface-tension proteins which are not as hydrophobic as liver-specific proteins, may for that reason interact with pristine C_{60} to a lesser extent, because of the surface-tension properties of the tissue. The interactions between pristine C_{60} and the body need therefore to be mapped as thoroughly as possible, in terms of all organs, and with a particular focus of investigating multiple toxicological mechanisms of action, given that pristine C_{60} are so unique, yet diverse, in their chemical properties. Based on the notion that different organs have different cell types, unique metabolic purposes and mechanisms, it is clear that each of the organs has a specific microscopic environment which responds different to "intruders."

In addition to having unusual chemical properties, fullerenes have also been shown to have catalytic properties. It was demonstrated that they can oxidize iso-butanol through an oxidative dehydrogenation (Liang et al. 2009). The ability of fullerenes to be catalytically active therefore opens a new field of toxicology, where the toxic agents conduct interfering biochemical activities, rather than being passive particles that block mechanisms of the body.

Given their potential interaction with several cellular factors, the unknown incubation time, and DNA-damaging properties (Yamakoshi et al. 2003; Markovic et al. 2007; Dhawan et al. 2006; Subach et al. 2009; Tokuyama et al. 1993; Zhang et al. 2009), their catalytic properties (Liang et al. 2009) and their membrane permeating properties (Nakagawa et al. 2011; Sadauskas et al. 2007; Jovanović et al. 2011) leading to cell death through the formation of Neutrophil extracellular traps (NETs), fullerenes need to be classified as nanotoxicological particles which need to be studied in all their available forms before they can be applied in cosmetics, medicinal and other industrial scopes. Particularly, given that recent results (Henry et al. 2011) argue that fullerenes do not induce significant damage in fish, the mechanism of toxicological damage as stated in other studies must be elucidated, particularly according to structure–function activity studies.

Structural and electronic properties of selected fullerenes

Due to the peculiar aspects of fullerenes, it is in place to also review a few crucial electronic properties of fullerenes. The purpose being that they can aid toxicologists and eco-toxicologists in designing schemes for prioritizing aspects and types of fullerenes for their investigations. This review is presented pedagogically, so that the toxicologist can identify the thread of importance between the electronic aspects, which can be studied using the tools of quantum chemistry, and their toxicological potential, both for in vitro and in vivo purposes.

Fullerenes have diameters of 1–10 nm giving them particularly hydrophobic qualities and making them highly baro- and thermotolerant (Jehoulet et al. 1992; Lee et al. 2010). The non-aromatic $sp2$ configuration of the carbon atoms allows them to have a unique chemical susceptibility to modifications and thereby to be the starting point for several product variants. Typical functional groups that are added to fullerenes are oxygen-, hydroxyl-, polyvinylpyrrolidone (Khairullin et al. 1997a) and methyl-ester moieties

(Hummelen et al. 1995). The addition of *some* polar groups aids the fullerenes to gain trans-membrane crossing properties, given their better interaction with aqueous environment outside the cell, avoiding aggregation (Bakry et al. 2007). Fullerenes have also been frequently ligated to metal ions to form metal complexes (Khairullin et al. 1997b; Jakubov and Mainwaring 2009; Yang et al. 2009; Lin et al. 2011). These examples highlight the potential of fullerenes to gain conducting properties (Jakubov and Mainwaring 2009), to generate nanohybrid ultra-thin films by cross-linking with titanium ions (Yang et al. 2009) and to function in photovoltaic units for generation of electricity (Lin et al. 2011).

The arrangement of the atoms in fullerenes makes it possible for them to interact with several species of molecules. Interaction sites at the carbon-only fullerene rings can be located on most carbon atoms, however, upon modifications, specific sites occur. For instance, the most favorable sites for nucleophilic attacks are the carbon atoms found at the junction between the 5-ringed carbons and the 6-ringed carbon atoms. At these sites, ligation with nucleophiles such as hydroxyl groups, may take place and such the reactions can be studied for instance through quantum chemistry methods. Other parts of pristine C_{60} molecule, particularly the *sp*2-carbons of the hexagonal arrangements, are more susceptible for interaction with electrophiles such as strong Lewis acids, for instance H^+ ions.

Electronic analysis of the fullerene in interaction with cellular factors

The molecular orbitals of pristine C_{60} are delocalized, as in benzene, giving it its classical aromatic character and making it suitable as conductors (Chen and King 2005; Rodríguez-Fortea et al. 2011). The reactivity of a molecule is often related to its band gap, which is the energetic difference between the lowest unoccupied molecular orbitals (LUMO) and the highest occupied molecular orbitals (HOMO).

In context with reactivity, it is noteworthy to account for the high polarizability of pristine C_{60} as well (Eilmes and Munn 2004). The polarizability of pristine C_{60} makes it susceptible to interactions with electrophilic or nucleophilic components, such as found in the cellular environment. A simple quantum mechanical analysis of the pristine C_{60} fullerene with and without an adjacent Zn^{2+} ion shows its polarization interaction with the divalent metal ion (Fig. 2). The analysis which was carried out using the Gaussian package (Frisch et al. 2003), using the B3LYP (p/6-31G*) method (Becke 1993; Lee et al. 1988; Vosko et al. 1980; Raghavachari et al. 1980; Rassolov et al. 1998), depicts the changes in the orbitals of the fullerene upon a non-covalent interaction with the divalent ion $Zinc^{2+}$.

When studying this interaction further, we observed that an interaction with the divalent metal ion induces a change in the chemical character of a fullerene modulating its aromatic character and binding the zinc ion covalently. This change illustrates that the chemical character of the pristine C60 shifts more from its benzene-like aromaticity to the anti-aromatic character of anti-benzene (annullene), a finding which denotes how divalent ions can modulate the fullerene's chemical character from the quantum level. At this stage, something of particular interest occurs, once bound the Zinc ion in an energetically favorable manner, the fullerene increases its chemical reactivity by 3.3 times: thereby having an increased potential of binding more ions (see Table 1). This creates a quantum effect that increases the reactivity of the fullerene molecule. By an increase in reactivity, the toxicological potential of the molecule may also increase.

To furthermore understand this potential electronic effect, we studied the effect of this molecule before and after binding the zinc, with typical cellular ions deriving from cellular reductants and oxidants, H^+ and OH^-. The complexation (binding) energies gave a clear indication on how spontaneous the fullerene would further absorb H^+ and OH^- ions, and as seen in Table 1, the interaction of fullerene with these ions is not only favorable energetically, but indicates a clear path of the absorption process of fullerene in the cell, binding divalent ions and protons in a manner that increases its reactivity 4.5 times. This finding indicates how the depletion of the energy in Kupfer liver cells in rat models (Nakagawa et al. 2011) might occur; fullerene depletes the proton storage in the inter-membrane region of the mitochondria, depleting the cell for energy. The binding of protons occurs in a favorable manner both with and without divalent ions such as zinc present.

The observed increase in reactivity may contribute to alter the toxicological properties of C_{60} inside the cell, behaving quantum toxicologically (Manzetti 2011) with the cellular chemical environment. These results suggest that divalent metal ions modulate the chemical reactivity of fullerenes by relocating the electrons in the complex, as indicated by the reduced band gap (Table 1) and the changed electronic configuration (Fig. 2).

A series of energy scans has also been performed to study the modulating activity of the Zinc ion on the actual binding process of the H^+ and OH^- ions (Fig. 2). This shows that the Zinc ion does not appear to affect substantially the complexation process of the H^+ and OH^- at a first glance, however, it indicates that it increases the reactivity of the fullerene, thereby binding new molecules more easily. The eventual direct effects of the zinc ion on the complexation of the H^+ and OH^- to the fullerene can

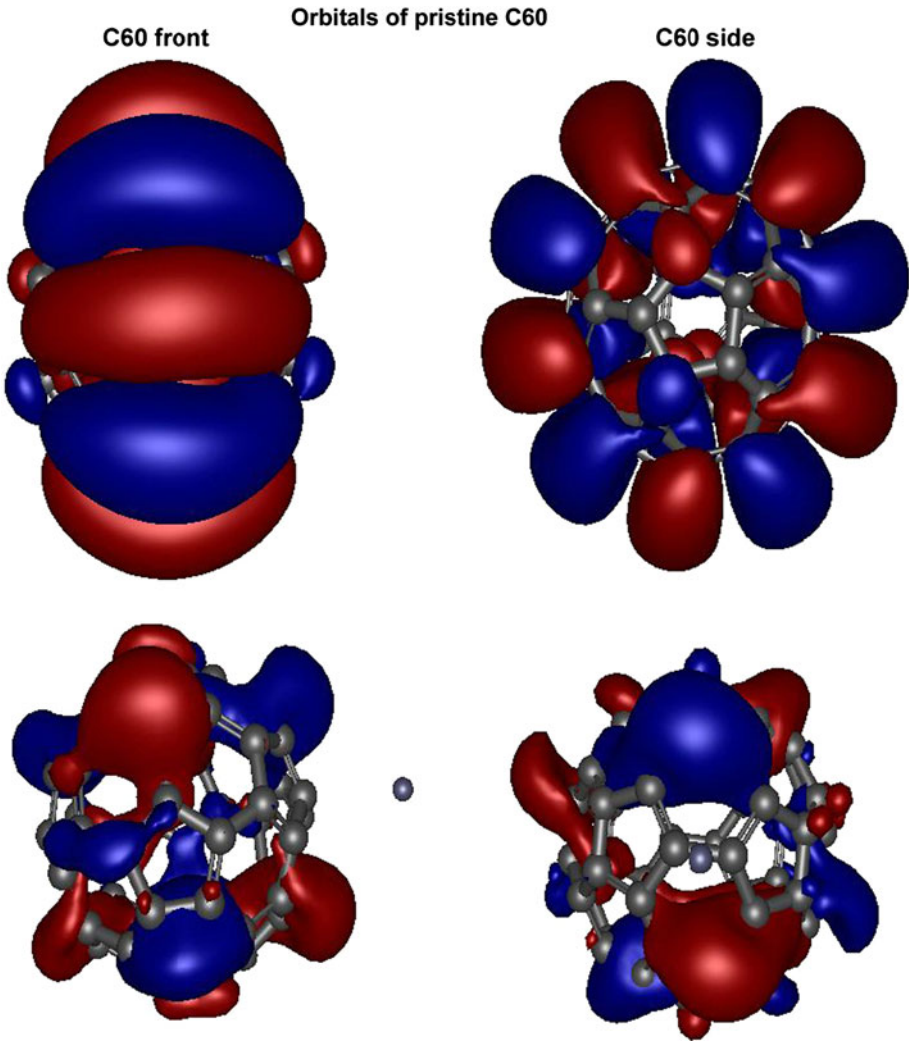

Fig. 2 Molecular orbitals of pristine C60, in their harmonized state, and adjacent to a strong polarizing Zinc ion. The molecular orbitals of fullerene seen from the front when affected by a Zn^{2+} ion: 2.54 Å away. Note how disoriented the orbitals become, indicating a significant delocalization of the electrons. This swift change in orbitals shows that fullerene tends to become anti-aromatic in the vicinity of Zinc (with localized surface electrons). Images generated in GabEdit (Allouche 2011). Quantum chemical calculations were performed with the Gaussian package (Frisch et al. 2003), using the B3LYP method with the 6-311G basis set for C60 and the 6-311G(d) for C60–Zn^{2+} complex (Becke 1993; Lee et al. 1988; Vosko et al. 1980; Raghavachari et al. 1980; Rassolov et al. 1998)

be furthermore investigated using tunneling energy calculations, which were omitted from the study for simplicity.

From a purely toxicological perspective, these findings should be viewed in the context of the strong oxidizing/reducing potential from various molecules in the cell, such as nicotinamide adenine dinucleotide phosphate (NADPH), flavin adenine dinucleotide (FADH2), nicotinamide adenine dinucleotide (NADH), vitamins and prosthetic groups. Fullerenes appear to behave as absorbers of chemical components in the cell, which is particularly relevant for the hydrogen ion which is donated from $NADPH^{+}$, $NADH^{+}$ and FADH2. These findings are compatible with the application of fullerene as an environmental remediator, binding toxic compounds for detoxification purposes in soil samples (Mauter 2008). As a cell intruder, however, fullerene appears to function as a biochemical absorber. In this context, the depletion of ATP in Kupfer cells (Nakagawa et al. 2011) can be explained by the fullerene targeting H^{+} ions, which are the driving force of the ATP synthesis (Voet and Voet 2010). This is thus an example where quantum chemical calculations can explain a toxicological phenomenon observed in vitro (Nakagawa et al. 2011).

Discussion

Many biochemical compounds in the cell, such as the mentioned cofactors, have a strong reactivity precisely in order to carry out their pivotal tasks as oxidants and reductants, and due to the electronic properties of fullerenes, may be of particular affinity to these nanoparticles. Further studies using quantum chemical methods may produce evidence that fullerenes can co-bind these compounds in the same manner as smaller chemical compounds, such as ions and metals. This knowledge may also serve to explain or hypothesize mechanisms of toxicological action by the fullerenes in particular cell types, by taking into account their unique biochemical signatures, for example, melatonin-producing cells in the brain. There are

Table 1 Results of quantum chemical calculations

	C_{60}	C_{60}–Zn^{2+}	C_{60}–H^+	C_{60}–H^+–Zn^{2+}	C_{60}–OH^-	C_{60}–OH^-–Zn^{2+}
Complexation energy (kcal/mol)	–	−244.144	−218.177	−330.642	−103.589	−509.694
HOMO (eV)	−5.985	−11.836	−9.100	−14.714	−1.766	−8.020
LUMO (eV)	−3.227	−11.007	−7.326	−14.112	−0.106	−7.410
E_{gap}(eV)	2.758	0.829	1.774	0.602	1.660	0.610
Reactivity (×C_{60} reactivity)	1	3.3×	1.5×	4.5×	1.7×	4.5×
Result	The C_{60} binds Zn2+ spontaneously and energetically favorable. By binding the Zn, the fullerene increases its reactivity by a factor of 3.3. Fullerene has thereby the ability to deplete the cell for divalent ions such as Zinc, in an energetically favorable manner		Fullerene binds both the Zn and the Hydrogen covalently. Fullerenes ability to bind NADPH/H+ and divalent metal ions represents a toxicological definition, where a mechanism of toxicity suggest a potential depletion of Zinc ions and protons in the cellular environment. The mitochondria, which rely on the high gradient of protons for energy generation, are particularly prone to quantum toxicity effects by the C_{60}. The highest increment in reactivity occurs also when a divalent ion such as Zinc is present, increasing the fullerenes reactivity by 4.5 times		Fullerene binds the OH^- covalently and the Zinc ion. Both the OH^- and the Zinc ion increase the reactivity of the fullerene, where the final complex, with both OH^- and Zn^{2+} bound reach the highest reactivity of 4.5 times the pristine fullerene	

Quantum chemical calculations of the three different arrangements; C_{60} w/without Zn^{2+}, C_{60}–H^+ w/without Zn^{2+} and C_{60}–OH^- w/without Zn^{2+}. The complexation (binding) energies are shown in kcal/mol. Each complexation energy results from the energy difference between the final complex (i.e., C_{60}–Zn^{2+}) and the energy of the components individually (energy of C_{60} and energy of Zn^{2+}). The HOMO and LUMO energies are in electron-volt (eV). Their difference and band gap delineates the reactivity of the molecular complex. The smaller the band gap, the higher the chemical reactivity

Table 2 Toxicology priorities for studies on fullerenes

Fullerene types	Toxicology topics	Remarks
Pristine C_{60}	Lipid-metabolomic pathways[a], brain development, Kupfer cell, liver cell studies	Pristine C_{60} are strongly hydrophobic. Given their nature, the fat metabolism and related functions can be prioritized for half-life studies for instance
Hydroxylated (solubilized) C_{60}	Respiratory and cardiological studies, kidney metabolism studies, blood-barrier studies	Solubilized C_{60} have a more hydrophilic character than pristine fullerenes. For this reason, the authors suggest focus on hydropathic bio-functions, where kidney and blood-barrier studies may be particularly relevant
C_{70}, C_{50}, C_{52}	Lipid-metabolomic pathways, brain development, teratogenic studies, hormone studies	The strong aromaticity of these fullerene variants (Chen and King 2005; Chen et al. 2004) should function to direct toxicologist to prioritize these as aromatic toxic compounds and thereby prioritize the very important topics of toxicological studies, embryo studies, brain tissue studies and organism-developmental studies
Large fullerenes (aerosol)	Respiratory-, pleural-liquid studies, bronchial studies	The case of larger fullerenes is known to promote more mediocre chemical properties (Chen and King 2005; Chen et al. 2004; Rodríguez-Fortea et al. 2011) in context with aromaticity and hydrophobicity, and these variants, increasing in molecular size, may therefore act more alike particles rather than reactive species
Small fullerenes	Cell culture studies, energy metabolism, blood-barrier-crossing studies	The documented reactivity of small fullerenes (Chen and King 2005; Chen et al. 2004; Rodríguez-Fortea et al. 2011) makes that these molecules have significant toxicological potential which indicates the need for cellular mechanisms studies, such as energy metabolism

The remarks are not exclusive to each other. Alternative directions are feasible

Table 3 Topic for pharmacological studies of fullerenes

Fullerene types	Pharmacology topics	Remarks
Pristine C_{60}	Cancer cell lysis, cancer therapies	The hydrophobic character of pristine C_{60} may serve various functions in decomposing membranes in isolated and selected tissues, such as in tumors
Hydroxylated (solubilized) C_{60}	Drug delivery, biomarker studies	The partial solubility of hydroxylated and solubilized fullerenes may be particularly relevant with biomarker functions in pharmacology
C_{70}, C_{50}, C_{52}	Cancer cell lysis, cancer therapies	The strong aromaticity of C_{70}, C_{50}, C_{52} can aid pharmacologist to work with particular emphasis on anti-tumor studies
Large fullerenes (aerosol)	Adsorption for detoxification purposes	Because of the large surface of these fullerenes, their high adsorptive potential may be applied in detoxification or adsorption purposes in pharmacology studies, in the same manner as Lanthanum tables are used to adsorb excess phosphate in nephritis patients (Gonzalez-Parra et al. 2011)
Small fullerenes	Selective cancer cell intoxication	The reactivity of small fullerenes (Chen and King 2005; Chen et al. 2004; Rodríguez-Fortea et al. 2011) makes them suitable as target-specific compounds which can be exploited in pharmacology

The remarks are not exclusive to each other. Alternative directions are feasible

Table 4 Topics for eco-toxicological studies of fullerenes

Fullerene types	Ecotoxicology topics	Remarks
Pristine C_{60}	Plant studies, soil microbial population studies, aquatic agglomeration studies. Life cycle studies	Because of the weakly-aromatic and agglomerating character of pristine C_{60}, the authors argue on that life cycle studies may be particular important, along with soil chemistry studies
Hydroxylated (solubilized) C_{60}	Co-contaminant-studies, algae-studies, tap water. Life cycle studies	The partial solubility of fullerenes accounts for a possibility of binding to various compounds, both hydrophilic and hydrophobic. This is particular can be included in concert with co-contaminant studies in ecological settings
C_{70}, C_{50}, C_{52}	Studies within confined ecosystems with emphasis on effects of strong aromaticity	Because of the aromaticity, the authors argue that C_{70}, C_{50}, C_{52} in particular can be evaluated in concert with cross-reactivity studies with nutritional sources within defined ecosystems, for instance small aquatic systems such as lakes
Large fullerenes (aerosol)	Decontamination of chemicals, soil chemistry studies	Large fullerenes may be exploited for efficient decontamination of aromatic compounds in ecosystems, because of their chemical properties and their chemical stability (Chen and King 2005; Chen et al. 2004; Rodríguez-Fortea et al. 2011)
Small fullerenes	Cross-reactivity studies with nutrition sources in confined ecosystems	The reactivity of these fullerenes (Chen and King 2005; Chen et al. 2004; Rodríguez-Fortea et al. 2011) points to the potential of interaction with nutrition sources in confined ecosystems

The remarks are not exclusive to each other. Alternative directions are feasible

therefore a series of studies that can be carried out using quantum chemical methods to reveal quantum toxicological effects. In this context, Tables 2, 3, 4 connects groups of fullerenes with specific biochemical, biologic properties to toxicological, pharmacological and eco-toxicological topics that are particularly important to study. Here, we account for that the shapes and sizes of fullerenes affect their chemical susceptibility where large differences occur upon increasing/decreasing the number of atoms in fullerenes.

Figure 3 depicts some ordinary molecular models of fullerenes, illustrating how these particles vary in size and shape, and because of this, also in chemical reactivity. For instance, the electronic properties are significantly different between small and larger fullerenes; the smaller they are, the more reactive they become, because of higher presence of pentagonal arrangements of the carbon atoms giving more localized *sp*2-orbitals (Chen and King 2005; Rodríguez-Fortea et al. 2011; Chen et al. 2004). The difference between the various fullerenes may in turn be so large, that some fullerenes, such as C_{60}, are even characterized as *not* being a strong π-aromatic compound (Chen and King 2005). This can be explained by the electron localization in fullerene C_{60}, which has a favorable energy in a non-π-aromatic configuration, given that the interior π-orbitals, interior of the fullerene cage, act magnetically in an opposite fashion to the exteriorly located π-electrons. Compared with the weakly-aromatic C_{60}, the C_{70}, C_{50} and

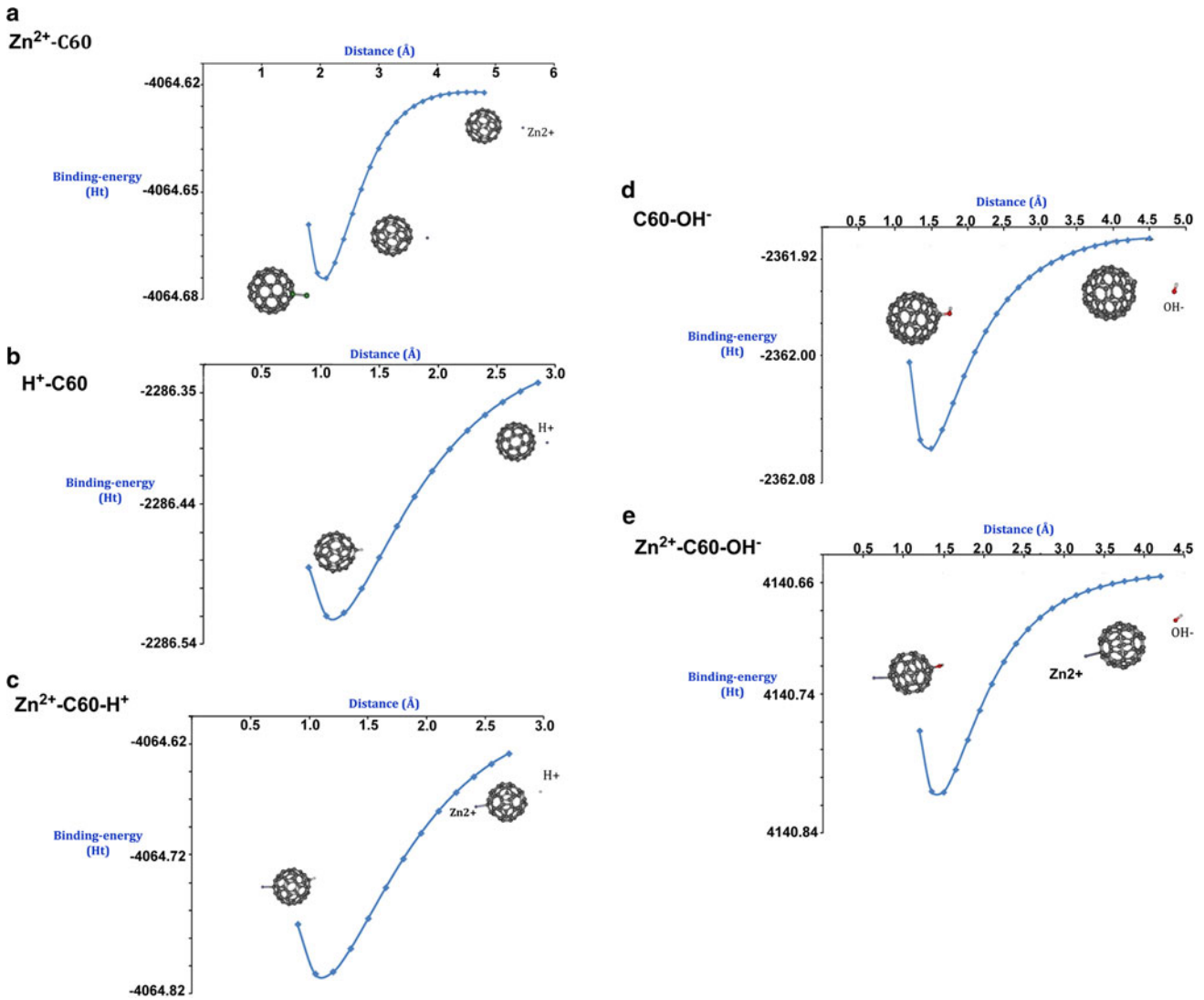

Fig. 3 Complex-formation energy scans. A series of scans of the energies, in Hartree energy units, of the complexes during complex formation. From *Left* to *right*, **a** C60 complexation with Zinc ion, **b** C60 complexation with the H^+ ion, **c** (C60–Zn2+) complexation to the H^+ ion, **d** C60 complexation with the OH^-. **e**, **c** (C60–Zn^{2+}) complexation to the OH^- ion. The binding of these two agents seemingly proceeds at the same rate of formation as without as a Zinc ion adjacent or bound. The Zinc ion shows, however, to increase the reactivity of the fullerene, which itself is a rate-accelerating value for general chemical reactions to occur (see Table 1)

C_{52} are characterized as being highly aromatic (Chen and King 2005). Therefore, two "structurally similar, but chemically different fullerenes" may be similar at first glance, but may have completely opposite magnetic properties and very different chemical properties and indeed act quite differently in vivo.

Yet other fullerenes, such as those with higher carbon number, have more "intermediate" aromatic properties (Chen and King 2005; Chen et al. 2004). Calculations show that the binding of larger fullerenes to other groups strongly affects their magnetic properties, yielding yet again a wide range of chemical properties to the fullerene family (Stephens et al. 1994). The configuration of fullerenes makes them therefore prone to chemical modifications and slight alterations of atomic numbers may give significant differences in chemical pathways of reactivity and toxicological mechanisms. Also, the various sizes of fullerenes and the modifications such as adducts or ionic species, for example, hexa- or deca-anions (C_{60}^{6-}, C_{60}^{10-}) have been demonstrated, but these are unlikely in vivo to yield a wide range of toxico-chemical aspects of fullerenes, and therefore, potential for interference with various biochemical factors.

The notion that various fullerenes may exert completely different toxicological actions is supported by studies showing that fullerenes with similar symmetry and number

of carbon atoms, that is, isomers may behave quite differently (Rodríguez-Fortea et al. 2011; Haddon and Pasquarello 1994; Knupfer et al. 1996). The changes in size, composition and fullerene similarities/dissimilarities therefore present important aspects to be accounted for in regard to toxicological analyses of fullerenes.

Conclusion

Fullerenes exert various modes of toxicological damage to bacteria, plankton, cells and multicellular organisms. Their action is primarily explained by three factors: (1) their ability to penetrate membranes, (2) their propensity to form aggregates, (3) their potent reactions with different biochemical compounds and (4) their ability to generate nanoparticles of different diameters based on the chemical environments, for example, hydrated, solubilized or aerosol. Fullerenes avoid lysosomes and may carry co-contaminants that can cross the blood–brain barrier. In order to explain some of these unique properties, a survey on the electronic properties of fullerenes has been carried out, along with a review of toxicological properties.

In particular, the discussed chemical changes that occur with the increase/decrease in cage size present pivotal aspects for nanotoxicological assessments in the future, in order to better predict, detect and measure toxicity. These studies can to a significant extent be aided by quantum chemical calculations so that bio-toxicological reactions can be better understood and predicted when working with nano materials. Much work on fullerenes therefore remains, and extensive toxicological studies are needed in order to determine the toxicological properties of fullerenes according to their chemical properties, size, composition and interaction with different types of organs and tissues in the body.

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