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Review

# Humic acids: Structural properties and multiple functionalities for novel technological developments

Bruna Alice Gomes de Melo, Fernanda Lopes Motta, Maria Helena Andrade Santana *

*Development of Biotechnological Processes Laboratory, School of Chemical Engineering, University of Campinas, 13083-852 Campinas, São Paulo, Brazil*

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ABSTRACT

Humic acids (HAs) are macromolecules that comprise humic substances (HS), which are organic matter distributed in terrestrial soil, natural water, and sediment. HAs differ from the other HS fractions (fulvic acid and humins) in that they are soluble in alkaline media, partially soluble in water, and insoluble in acidic media. Due to their amphiphilic character, HAs form micelle-like structures in neutral to acidic conditions, which are useful in agriculture, pollution remediation, medicine and pharmaceuticals. HAs have undefined compositions that vary according to the origin, process of obtainment, and functional groups present in their structures, such as quinones, phenols, and carboxylic acids. Quinones are responsible for the formation of reactive oxygen species (ROS) in HAs, which are useful for wound healing and have fungicidal/bactericidal properties. Phenols and carboxylic acids deprotonate in neutral and alkaline media and are responsible for various other functions, such as the antioxidant and anti-inflammatory properties of HAs. In particular, the presence of phenolic groups in HAs provides antioxidant properties due to their free radical scavenging capacity. This paper describes the main multifunctionalities of HAs associated with their structures and properties, focusing on human health applications, and we note perspectives that may lead to novel technological developments. To the best of our knowledge, this is the first review to address this topic from this approach.

**Contents**

## 1. Introduction

Humic acids (HAs) are macromolecules that comprise humic substances (HS), which are organic matter distributed in terrestrial soil, natural water, and sediments resulting from the decay of vegetable and natural residues [1]. Commercial HAs are extracted from peat and coal, which are non-renewable sources of carbon. Recently, it was demonstrated that HAs could be produced by fermentation using the empty fruit bunch (EFB) of palm trees as a substrate, which is a natural and sustainable resource [2]. Furthermore, chemical synthesis can also be used to produce HAs through polymerization/condensation reactions [3,4].

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* Corresponding author.
*E-mail address:* mariahelena.santana@gmail.com (M.H.A. Santana).

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HAs are the fractions of HS that are soluble in alkaline media, partially soluble in water and insoluble in acidic media [5,6]. This classification parameter may vary with the HAs composition, pH, and ionic strength [7]. Due to their amphiphilic character, HAs form micelle-like structures, called pseudo-micelles in neutral to acidic conditions [8,9]. This property has been explored for use in pollution remediation [10–14] and to increase the water solubility of hydrophobic drugs [15,16].

HAs contain different functional groups whose quantities depend on the origin, age, climate, and environmental conditions of extraction/production of the HAs [5,17,18]. The various functions of HAs are mainly attributable to the phenol and carboxylic acid functional groups [19], which allow the deprotonation of OH/OOH. This situation provides HAs with many capabilities, such as the improvement of plant growth and nutrition [20–23], complexation with heavy metals [24], and antiviral and anti-inflammatory activity [25–29]. In addition, the presence of phenols, carboxylic acids and quinones in the structure of HAs is related to their antioxidant, antimutagenic/desmutagenic and fungicidal/bactericidal activities [30–33].

The use of HAs is quite consolidated in agriculture [34,35] and pollution remediation [36,37]. Recently, review articles by Calvo et al. [38] and Canellas et al. [39] addressed the specific effects of HAs on plants and their role on the plant growth, yield and nutrient uptake. Meanwhile, Tang et al. [40] and Sun et al. [41] discussed the importance of HAs in the treatment of water and waste gas, respectively. In medicine, HAs have been studied for the treatment and prevention of diseases [31,42–44]. The main medical aspects and applications of HAs were described by Klöcking & Helbig [27]. More recently, van Rensburg [45] highlighted the anti-inflammatory properties of HS in a mini-review. However, the application of HAs in the pharmaceutical and cosmetic fields has not been well explored despite their great potential, such as in the solubilization of hydrophobic drugs, in UV–visible absorption and as an antioxidant [15,16,30,46]. In previous work, we demonstrated that HAs interact with Pluronic F127 (PF127) to form stable nanoparticles, which can be used for pharmaceutical applications as-is or after entrapping nonpolar drugs [47]. In 2005, Peña-Méndez et al. [48] noted the applications of HS in environmental and biomedical applications. However, there is a lack of published studies that bring together all the functional effects of HAs related to their structural properties, as well as their toxicity and applications, especially in pharmaceutical and cosmetic areas.

Therefore, this review aims to be the first to present the multifunctionalities of HAs, associating them with their structure and properties, and note novel technological developments. Moreover, the role of HAs in human health is highlighted.

## 2. HAs structure and composition

The chemical composition of HAs can vary according to geographical origin, age, climate and biological conditions, making the precise characterization of these substances difficult [49]. Their molecular weights are in the range of 2.0 to 1300 kDa [17], and they contain many functional groups, as shown in Fig. 1. HAs are mainly composed of phenolic, carboxylic acid, enolic, quinone, and ether functional groups but may also include sugars and peptides [5]. However, the phenolic and carboxylic groups are more prevalent in HAs structures. The huge structure of an HA molecule is composed of hydrophilic portions, consisting of OH groups, and hydrophobic portions, consisting of aliphatic chains and aromatic rings.

The phenolic and carboxylic groups are responsible for the weak acid behavior of HAs. The total acidity (phenolic + carboxylic group acidity) of the compounds extracted from soil, water, and geologic deposits was found to be approximately 6 meq $g^{-1}$ [50].

Quinones are electron-accepting groups and are responsible for the production of reactive oxygen species (ROS). They are reduced to semiquinones, which are stabilized by their aromatic rings and further reduced into hydroquinones, which are even more stable (Fig. 2) [51].

Aeschbacher et al. [52] evaluated the electron-accepting (quinone) and electron-donating (phenol) moieties in HAs obtained from different sources. The origin and age of these substances were found to have a direct effect on their redox properties. $^{13}C$ NMR analyses have shown that aquatic HAs have higher numbers of electron-donating and lower numbers of electron-accepting moieties than terrestrial HAs, as verified by Scott et al. [51]. The authors supported the hypothesis that the phenolic groups in HAs slow the oxidative transformation of quinones, increasing their permanence in oxic environments.

There is an agreement in the literature on the average elemental composition of HAs extracted from different sources, including commercial HAs, which are approximately 50% C, 35% O, and 5% H, with the remaining percentage distributed between N and S, as shown in Table 1.

## 3. HAs properties

The main properties of HAs, such as solubility, pH dependence, interaction with hydrophobic groups, and metal chelation, are related to their structure, i.e., their amphiphilicity and the different functional groups that comprise each molecule. Table 2 shows the functional effects of HAs used in different applications areas, relating them to their structural properties.

### 3.1. Solubility and pH dependence

HAs are generally considered soluble in neutral to alkaline conditions [5]. This property varies with the chemical composition of these substances and thereby with their origin.

In alkaline media, phenolic and carboxylic groups are deprotonated, and the repulsion of these negatively charged groups causes the molecules to assume a stretched configuration. Upon decreasing the pH, the functional groups are protonated, and the effects of repulsion

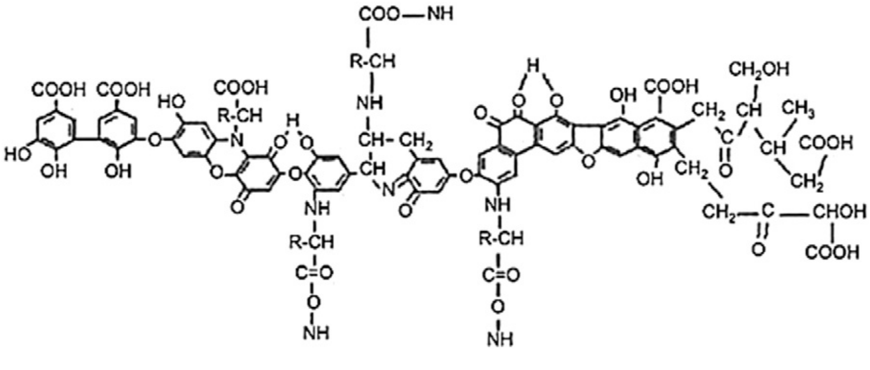

**Fig. 1.** Model of HA structure.
Figure adapted with permission from Mirza et al. [15]. Copyright (2011) Taylor & Francis Ltd. (www.tandfonline.com).

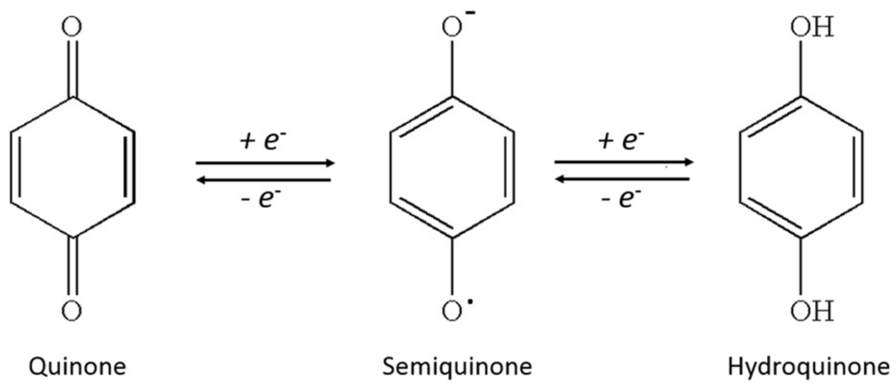

**Fig. 2.** Quinone, semiquinone, and hydroquinone structures and their oxy-redox reactions.

are minimized, causing the molecule to adopt a coiled and compact structure. In this stage, the hydrophobic portions are in the interior of the structure, and the hydrophilic portions are in contact with the aqueous medium. This behavior is responsible for the detergent characteristics of HAs, their micelle-like organization, and the decrease of the superficial tension. These molecules form aggregates on an intramolecular level, followed by intermolecular aggregation and ultimately precipitation, as suggested by von Wandruszka [9] and shown in Fig. 3.

Prado et al. [89] have noted that pH is related to not only the solubility but also the stability of aqueous suspensions of HAs. A diffuse electric double layer is formed around the charged particles, protecting them and allowing the system to become uncharged. Moreover, the ion concentrations determine the particles' charge protection, which is greater for lower ionic strength systems because the ionic species will have a stronger interaction with the electric layer than with the solvent molecules.

At neutral pH, the solubilization of HAs is only partial. Although HAs can be found dissolved in water in nature, not all isolated solid preparations will dissolve. Klučáková & Pekař [6] proposed different dissolution mechanisms for a lignite HAs–water suspension in which the solid has insoluble and soluble fractions. Because HAs are a weak polyelectrolyte, they can exist in water as dissolved molecules and in the dissociated form (Eq. (1)). This mechanism corresponds to the soluble fraction of HAs. The insoluble fraction interacts with the environment through the surface and acts as an ion-exchanger by releasing $H^+$ ions into solution while anions remain insoluble (Eq. (2)).

$$\mathrm{HA_{(S)}} \leftrightarrow \mathrm{HA_{(aq)}} \leftrightarrow \mathrm{H^+}_{(aq)} + \mathrm{A^-}_{(aq)} \tag{1}$$

$$\mathrm{HA_{(S)}} \leftrightarrow \mathrm{H^+}_{(aq)} + \mathrm{A^-}_{(S)} \tag{2}$$

Therefore, the dissolution of HAs molecules in water is more complex than that of common sparingly soluble solids because HAs are not a single component but a mixture of components, only some of which are soluble in water.

**Table 1**
Elemental compositions of HAs extracted from different sources.

| HAs | C (%) | H (%) | N (%) | O (%) | S (%) | References |
|---|---|---|---|---|---|---|
| Commercial (Sigma-Aldrich) | 55.6 | 5.5 | 4.5 | 34.4 | 1.2 | [33] |
| Soil IHSS standard | 58.1 | 3.7 | 4.1 | 34.1 | – | [53] |
| Peat soil | 50.4 | 4.9 | 2.8 | 39.1 | 0.7 | [44] |
| Sediments | 43.7–53.8 | 4.1–5.8 | 3.5–6.2 | 31.1–37.1 | – | [45] |
| Sewage sludge | 52.8 | 6.8 | 6.5 | 33.9 | 0.1 | [54] |
| EFB* | 56.3 | 5.7 | 4.4 | 32.9 | 1.2 | [32] |
| River | 51.2 | 4.7 | 2.6 | 40.4 | 1.9 | [55] |
| Leonardite | 63.8 | 3.7 | 1.2 | 31.3 | – | [53] |

* EFB — Empty Fruit Bunch.

### 3.2. Amphiphilic character

Many studies in the literature have reported the use of HAs as an alternative for solubilizing nonpolar substances in aqueous environments [10–14], with greater emphasis on their interaction with polycyclic aromatic hydrocarbons (PAHs), which are relatively insoluble in water and exhibit toxicity and carcinogenicity. The interactions between HAs and these nonpolar contaminants are noncovalent, and these compounds are solubilized at the hydrophobic core of HAs pseudo-micelles [24].

#### 3.2.1. Solubilization of hydrophobics

Lassen & Carlsen [11] verified the effect of dissolved commercial HAs in solubilizing solid fluorene and its heteroanalogs: carbazole, dibenzofuran, and dibenzothiophene. The PAHs were dispersed in water containing different amounts of HAs, and an increase in the apparent aqueous solubility of all PAHs that was also affected by HAs concentration was observed. The solubility of fluorene increased by 50% in the presence of 0–200 mg $L^{-1}$ HAs, while the solubility of dibenzothiophene increased by more than 600% in the presence of 0–100 mg $L^{-1}$ HAs. Furthermore, it was found that HAs adsorb to dispersed PAHs particles, but this sorption competes with the dissolution mechanism. Thus, lower sorption leads to a stronger solubility effect of HAs on the PAHs particles. The constant of interaction, $K$, will vary among PAHs and decreases as the concentration of HAs in the solution increases. This behavior may be related to changes in the conformation of the HAs structure, which tends to coil up with increasing HAs concentration, as also found with decreasing pH and increasing ionic strength [90]. This coiling restricts the hydrophobic interaction between the two substances because the nonpolar fraction of HAs is located in the inner portion of the structure.

More recently, Tejeda-Agredano et al. [12] investigated the influence of HAs on the biodegradation of PAHs by microorganisms. The availability of these compounds to microorganisms is affected by their low water solubility, which interferes with their dissipation in polluted soil and sediments. Their study showed that the degradation of pyrene at a concentration above its solubility was significantly faster in the presence of HAs. On the other hand, HAs can inhibit cell adhesion on PAHs surfaces, limiting the biodegradation process.

In the medical, pharmaceutical, and cosmetic areas, different strategies for increasing the aqueous solubility of nonpolar compounds have been explored recently using such carriers as lipid nanoparticles [91], liposomes [92], cyclodextrins [93], and polymeric particles [94]. In addition to solubilizing hydrophobic compounds, HAs have surfactant characteristics, which makes them a potential new technology for cosmetic and drug delivery. Few studies in the literature have reported

**Table 2**
Applications of HAs and their functional effects related to their structures.

| HAs technological application | Functional effects | Structural properties | References |
|---|---|---|---|
| Pollution remediation | Chelates heavy metals | OH/OOH deprotonation | [24,36,37,40,41,56–59] |
| | Solubilizes hydrophobic pollutants | Amphiphilic character | [10–14,60,61] |
| Agriculture | Plant growth and nutrition | OH/OOH deprotonation | [22,23,34,35,39] |
| | Plant growth and nutrition | Production of active oxygen (ROS) | [38,62–64] |
| | Bactericidal | Production of active oxygen (ROS) | [32,65] |
| | Fungicidal | Production of active oxygen (ROS) | [33] |
| Medicine | Antiviral | OH/OOH deprotonation | [25–27,29,46,66] |
| | Anti-inflammatory | OH/OOH deprotonation | [27,28,45,67–70] |
| | Antimutagenic/desmutagenic | Presence of OOH | [31,44,71–73] |
| | Wound healing | Production of active oxygen (ROS) | [27,42,45,74,75] |
| | Cancer therapy | Production of active oxygen (ROS) | [42,76–79] |
| | Prion disease therapy | OH/OOH deprotonation | [38] |
| Pharmaceutical and cosmetic | UV–vis protection | UV–vis absorption | [3,46,80] |
| | Antioxidant | Presence of OH | [30,52,81–85] |
| | Drug solubilizer and carrier | Amphiphilic character | [15,16,47,86–88] |

the use of HAs as an alternative means of solubilizing, loading, and consequently enhancing the bioavailability of hydrophobics. Such studies will be discussed further below.

### 3.3. Binding cationic metals

The ability of HAs to bind cationic metals and form complexes makes them useful in various applications, such as the transport of micronutrients from the soil to plants [20], the removal of heavy metals from soil and water [24], the inhibition of the formation of free radicals by metal catalysis [95], reduction, and stabilization of metal nanoparticles [96].

The role of metal ions in solution is the same as that of $H^+$ ions, namely, charge neutralization, and the higher the charge, the more effectiveness the cation is in the formation of pseudo-micelles. Furthermore, multivalent cations interact with phenolic and carboxylic groups on adjacent chains, enhancing pseudo-micellar domains and the detergent effect. The mechanism of this interaction proposed by von Wandruszka [9] is as follows. The interaction between HAs molecules and metal cations is initially entirely electrostatic, and the cations move to their thermodynamically preferred locations within the structure. This process forms spherical HAs-metal complexes, as shown in Fig. 4. Studies on HAs-metal binding have shown that this interaction varies according to the metal and is influenced by the metal concentration and the origin, molecular weight, and concentration of HAs [24,97].

Christl et al. [97] demonstrated that the binding capacity between HAs and metals is associated with the molecular size of HAs. $^{13}C$ NMR analysis verified that HAs fractions with lower molecular weight have the highest number of phenolic and carboxylic groups and are therefore the fractions that can bind metals most efficiently. In another work, Christl & Kretzschmar [99] observed that higher concentrations of metal could improve the effectiveness of the interaction. At high concentrations of $Cu^{2+}$, the binding capacity of HAs was improved, especially in smaller molecules. In the same work, protonation assays were performed using $Cu^{2+}$, and the $H^+/Cu^{2+}$ exchange ratios of HAs suggested that Cu and HAs bind as monodentate and bidentate complexes. However, these characteristics vary according to the metal being complexed.

Yates & von Wandruszka [24] verified the affinity of leonardite HAs with different metals by retention in an HAs-packed column, finding that $Pb^{2+}$ and $Cu^{2+}$ had the greatest affinity for the column and that $Mg^{2+}$ had the lowest. The low retention of $Mg^{2+}$ is attributed to the low availability of HAs sites to this ion because of its large radius, which is the largest among the cations tested.

The ability of HAs to bind metals and form complexes, enabling their use as a pollution remedy by removing heavy metals from water and soil, is a fairly recent topic in the literature [56–59]. However, if the environment is acidic, which is typical of metal-polluted water, the solubility of HAs is reduced, which interferes in the formation of HAs-metal complexes.

## 4. Role in human health

Relative to their applications in agriculture and pollution treatment, there are few discussions of the use of HAs for the benefit of human health and wellness in the literature. In the following section, we will consider the effects and mechanisms of HAs in medical, pharmaceutical and cosmetic contexts as well as their toxicity to the human organism.

### 4.1. Medicine

Several works in the literature have studied the medicinal properties of HAs, which have been reported to be strong allies in the treatment of many diseases. The antiviral activity of HAs was observed against many viruses, such as cytomegalovirus (CMV), vaccine viruses, and human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2) [25,27,29]. HAs molecules, which assume a negative charge in neutral to basic media, can inhibit virus replication by binding cationic domains of the virus, which are necessary for virus attachment to the cell surface [29]. The anti-HIV activity of these substances was demonstrated through inhibition of the infectivity of in vitro human lymphocytes, in addition to blocking the formation of syncytia between infected and non-infected lymphocytes [26]. van Resburg & Naude [67] have demonstrated

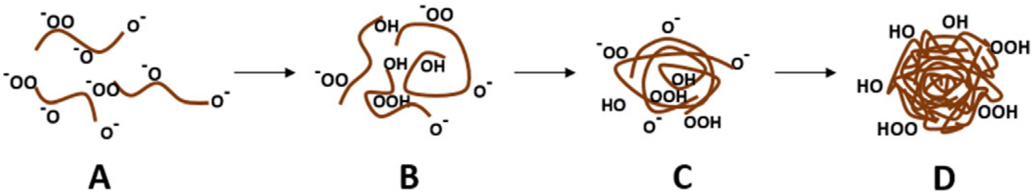

**Fig. 3.** Behavior of HAs molecules in alkaline conditions and the aggregation process upon pH reduction. Alkaline pH: charge repulsion (A). Decreasing pH: intramolecular aggregation (B). Decreasing pH: intermolecular aggregation (C). Acidic pH: precipitation (D).

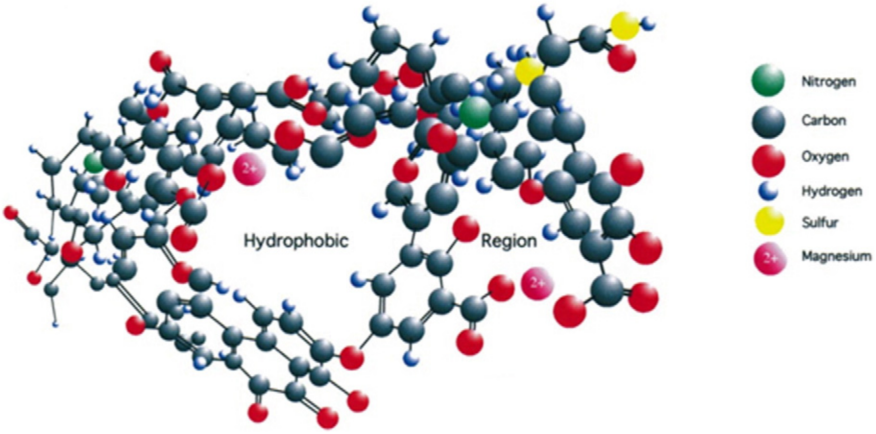

**Fig. 4.** Interaction of $Mg^{2+}$ ions with an HA molecule and its folding around the hydrophobic region.
Figure adapted with permission from Engebretson & von Wandruszka [98] Copyright (1994) American Chemical Society.

that potassium humate inhibits the production of inflammatory cytokines (TNF-α, IL-1β and IL-6) due to the binding properties of HS, and Junek et al. [28] have shown the bimodal effect of HAs on (LPS)-induced TNF-α release in human U937 cells. TNF-α is a cytokine with an important protective role against microbial effects, but at high levels, it is associated with many inflammatory diseases [100,101]. It was shown that at low concentrations of HAs, the TNF-α release is enhanced (pro-inflammatory effect), whereas at high concentrations ($>100\ \mu g\ ml^{-1}$), the release is reduced approximately 10-fold (anti-inflammatory effect). The authors attributed the bimodal effect of HAs to the presence of negatively charged functional groups because it is known that other polyanionic compounds induce changes in cytokine production.

HAs are also recognized as inhibitors of mutagenesis, possessing an antimutagenic activity that blocks the mutagenesis process inside the cell and a desmutagenic activity that inhibits mutagenesis outside the cell [44]. Ferrara et al. [31] have investigated the capacity of HAs to reduce the mutagenicity of mitomycin C (MMC) in the human lymphoblastoid cell line TK6. A significant desmutagenic activity in cells treated with a combination of HAs (leonardite and soil HAs) was verified by the induction of micronuclei (MN), whereas the antimutagenic activity was observed in a more limited way. In both cases, higher efficiency was observed at higher HAs concentrations, and the results varied with the type of HAs used. The authors related the biological activity to the concentration and composition of HAs, which changes according to the origin, age, and biological conditions of the HAs, as already mentioned.

Prion diseases are a group of neuropathies caused by a conformational modification in the structure of prion proteins (PrP). Normal PrP ($PrP^{C}$) are induced by a modified protein ($PrP^{SC}$) that changes their conformation from α-helical motifs to β-sheet secondary structures through a posttranslational process [102]. In Legname et al. [43], it was found that HAs could eliminate $PrP^{SC}$ infectivity from chronically infected living cells. The mechanism proposed by the authors is direct binding between HAs and $PrP^{C}$ blocking the conversion reaction from the normal PrP to the misfolded one.

The ability of HAs to bind metals was explored in medicine in a study by Litvin & Minaev [96]. The group synthetized silver nanoparticles (AgNPs) coated with synthetic HAs by electrostatic interactions to obtain a combination of biologically active nanoparticles for medical applications. It was verified that HAs were able to stabilize AgNPs via their repulsive forces for a long period of time (1 year). In addition, the antibacterial abilities of HAs enhance this effect on the AgNPs, suggesting that these conjugated nanoparticles have great potential in the preparation of pharmaceuticals.

HAs can have a positive effect on wound healing and cancer therapy, as suggested by Jurcsik [42]. The healing process requires extra oxygen, and this demand appears in the first minute after wounding due to phagocytosis, the main event in wound healing process, which is very oxygen-consumptive [42]. Semiquinones are able to produce ROS through different mechanisms, as shown in Fig. 5. In the presence of molecular oxygen, semiquinones produce superoxide ions, which are converted to hydrogen peroxide in the presence of superoxide dismutase. The superoxide ions produce hydroxyl radicals by reacting with transition metals (Fenton reaction) or hydrogen peroxide (Harber–Weiss reaction).

Quinones are widely studied in cancer therapy because ROS cause oxidative stress and induce apoptosis in cancer cells through DNA fragmentation and can also act as an intracellular signal of the apoptosis cascade. Moreover, quinones directly interfere in the apoptosis of normal and cancer cells in a concentration-dependent manner [76–79, 103].

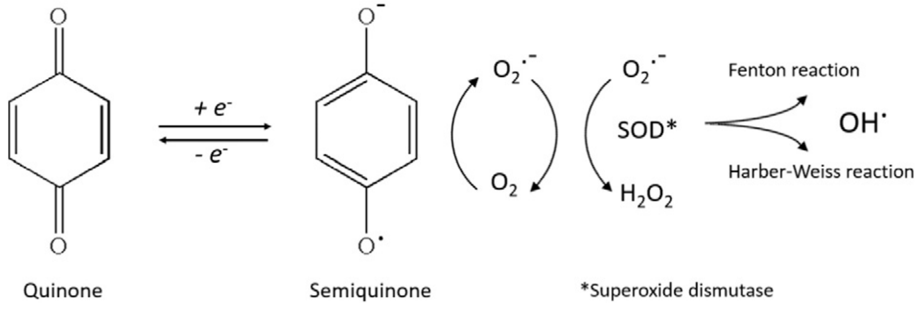

**Fig. 5.** Redox cycle of quinone.

### 4.2. Pharmaceutical and cosmetic areas

Although studies involving HAs in pharmaceutical and cosmetics applications remain scarce, the results note the potential of HAs as functional agents in the prevention or treatment of various diseases.

HAs *in natura* may be used in sunscreen, anti-aging, and skin care products in general due to their ability to absorb in the UV–visible range. Klöcking et al. [46] studied the potential of HAs as a component of lipsticks to prevent the reactivation of the *herpes simplex* virus by UV-light. It was shown that HAs in concentrations higher than 100 μg $ml^{-1}$ were able to protect U937 cells from UV-induced damage. These characteristics make HAs viable as components of functional lipsticks.

The antiviral activity of HAs could also be explored in cosmetics as a component of facial masks, which are used for the prevention of viral reactivation after a chemical facial treatment, as described by Wollina [104].

The phenolic groups in HAs act as electron-donating agents, scavenging free radicals and preventing chain reaction initiation. In addition, they are able to chelate metals, particularly iron and cooper, inhibiting the formation of free radicals by transition metal catalysis, controlling lipid peroxidation and DNA fragmentation [30,52]. These antioxidant properties are also useful in cosmetic and pharmaceutical applications.

Khil'ko et al. [30] have evaluated the antioxidant capacity of HAs from brown coal through their behavior in inhibiting radical-chain oxidation process of cumene and ethylbenzene initiated by azobisisobutyronitrile (AIBN) and dimethyl sulfoxide (DMSO). It was verified that the rate of oxygen absorption decreased significantly in the presence of HAs, and at high concentrations (10 g $L^{-1}$), the oxidation process was completely halted. Adam & Needham suggested the use of HAs derivatives as natural antioxidants for food preservatives but with potential use for a variety of purposes, such as in cosmetic applications and as nutritional supplements. These derivatives were obtained by reductive cleavage techniques and were more efficient and cost effective than other food antioxidants.

Indirectly, HAs could act as solubilizing agents, carrying pharmaceutical and cosmetic active ingredients in their micelle-like structures to enhance their water solubility. Carbamazepine (CBZ) is an antiepileptic drug that is practically insoluble in water and therefore has a poor bioavailability. Mirza et al. [15] evaluated the water solubility, release, and anticonvulsant activity of CBZ complexed with HAs. The solubility of the complex was greatly increased compared to that of the free drug and that of the drug release conducted in a dialysis bag. The anticonvulsant activity was studied in mice using the maximum electroshock seizure (MES) experiment, and the potency of CBZ-HAs was threefold higher than that of the free drug.

Complexes of β-carotene and HAs were synthesized by Martini et al. [16] to increase β-carotene water solubility. These carotenoids have important biological activities, including antioxidant properties and as a precursor of vitamin A, but their hydrophobicity restricts their use in the pharmaceutical, cosmetic, and food fields. The authors showed that the water solubility of β-carotene was strongly increased by complexation to HAs, and its stability towards light irradiation was improved by approximately 60%.

Ghosal [86] and Khanna et al. [87] developed delivery systems from HSs for active ingredients (pharmaceutical, nutritional, and cosmetic) with low solubility. The systems consisted of complexes between HSs and drugs produced by hydrophobic bonding, covalent bonding, or chelation. These systems were capable of increasing drug solubility, permeability, and bioavailability and were suitable for topical or oral administration. Therefore, HAs are a promising matrix for the incorporation of bioactive ingredients in nano- or microstructures.

### 4.3. Toxicity

HAs toxicity is recognized as being remarkably low [105–107]. Dermal tests conducted on both rats and rabbits revealed no abnormalities in acute or chronic toxicity studies with HS preparations, and no local irritancy was observed [105]. The effects of ocular irritation caused by HAs were also evaluated by Hen's Egg Test-Chorion Allantoic Membrane (HET-CAM) testing by Wiegleb et al. [108], and irritation in mucous membranes and skin was not detectable at HAs concentrations up to 10%. Sato et al. [44] conducted mutagenic tests using *Salmonella typhimurium* TA100 and TA98, and mutagenic effects were not observed for HAs preparations.

Nonetheless, the ROS present in HAs could mediate toxicity at certain concentrations [109,110]. Some works in the literature have reported HAs as being toxic to many mammalian cells [111–113] and contributing to Blackfoot disease [114]; both of these activities are related to ROS.

Nevertheless, the phenolic portions of HAs are able to slow the oxidative transformation of quinones [52]. HAs have a “buffering effect”, which means that they are able to produce and to bind ROS [42]. This finding is in agreement with the view that HAs have great potential as natural antioxidants despite the presence of quinone groups.

## 5. Novel technological developments

HAs have substantial potential to be used in pharmaceutical and cosmetics areas because they can act directly or indirectly in the prevention and remediation of many complications of the human body.

These functional effects could be improved through the interaction of HAs with surfactants [115,116]. In previous work, we demonstrated that HAs were able to interact with the nonionic surfactant PF127 by amphiphilic and electrostatic interactions due to their amphiphilic character and their potential to assume a negative charge by deprotonation of the OH/OOH groups [47]. The interactions formed stable and spherical HAs-PF127 nanoparticles with a highly hydrophobic core, which can be used for pharmaceutical applications as-is or after entrapping nonpolar drugs, increasing their water solubility and bioavailability.

## 6. Conclusions

HAs have been widely explored for several years for their benefits in agriculture and pollution remediation. They are known to enhance plant growth and nutrition and act as soil bactericidal and plant fungicidal agents, and they can be used to remove pollutants from water and soil. In medicine, they can act as antiviral and anti-inflammatory agents; have uses in wound healing, cancer and prion disease therapy; and exhibit antimutagenic/desmutagenic potential. In pharmaceutical and cosmetic areas, the use of HAs is recent but very promising. They are known to protect against UV–vis radiation and can act as antioxidants. Further applications include their use as solubilizing agents and for transporting hydrophobic active compounds, two applications that may be improved by their administration as HAs-surfactant nanoparticles. This novel technology will enable the production of stable HAs nanoparticles, with a large hydrophobic core that could be used for the encapsulation of nonpolar drugs, improving their delivery and bioavailability.

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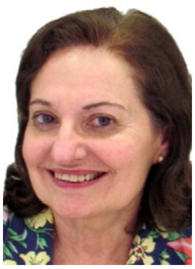
**Bruna Alice G de Melo** B.S. in Chemistry at the University of Campinas, Brazil (2011). Specialization in Material Sciences and Nano Objects at Pierre and Marie Curie University, France (2013). Presently working towards a master's degree in Chemical Engineering at the University of Campinas, Brazil, with emphasis on the production and characterization of humic acid nanoparticles for pharmaceutical applications.

**Fernanda Lopes Motta** B.S. in Food Engineering at the University of Viçosa (2007). Specialization in Biotechnology at the Institut National Polytechnique de Lorraine (INPL) France (2006), Master's Degree in Food Engineering at the University of Campinas (2010). PhD (2013) and postdoctoral research (2013-2015) in Chemical Engineering at the University of Campinas. Experience in biotechnology, with emphasis on fermentation, enzymatic process and nanoparticle production.



**Maria Helena A Santana** B.S. in Industrial Chemistry at University of Sergipe (1972). B.S. in Chemical Engineering from the University of Maringa (1976). Master's in the Science and Technology of Food at the University of Campinas (1980). DSc in Mechanical Engineering at the University of Campinas (1988). Postdoctoral research in Chemical Engineering at North Carolina State University (USA) (1993). Presently is a full professor at the School of Chemical Engineering of the University of Campinas, Brazil. Coordinates the Laboratory for Development of Biotechnological Processes. Elected member of the National Academy of Engineering (2014).