Published in IET Nanobiotechnology
Received on 22nd October 2010
Revised on 13th January 2011
doi: 10.1049/iet-nbt.2010.0033

ISSN 1751-8741

Polysaccharides and phytochemicals: a natural reservoir for the green synthesis of gold and silver nanoparticles

Y. Park[1] *Y.N. Hong*[2] *A. Weyers*[3] *Y.S. Kim*[2] *R.J. Linhardt*[3,4]

[1]*College of Pharmacy, Inje University, 607 Obang-dong, Gimhae, Gyeongnam 621-749, Republic of Korea*
[2]*Natural Products Research Institute, College of Pharmacy, Seoul National University, 599 Gwanangno, Gwanak-gu, Seoul 151-742, Republic of Korea*
[3]*Department of Chemistry and Chemical Biology, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, 110 8th Street, Troy, NY12180, USA*
[4]*Departments of Chemical and Biological Engineering, Biology, and Biomedical Engineering, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, 110 8th Street, Troy, NY 12180, USA*
E-mail: linhar@rpi.edu

Abstract: Currently, sustainability initiatives that use green chemistry to improve and/or protect our global environment are becoming focal issues in many fields of research. Instead of using toxic chemicals for the reduction and stabilisation of metallic nanoparticles, the use of various biological entities has received considerable attention in the field of nanobiotechnology. Among the many possible natural products, polysaccharides and biologically active plant products represent excellent scaffolds for this purpose. Polysaccharides have hydroxyl groups, a hemiacetal reducing end, and other functionalities that can play important roles in both the reduction and the stabilisation of metallic nanoparticles. Among the various categories of compounds in plants that have potent biological activities, phytochemicals are emerging as an important natural resource for the synthesis of metallic nanoparticles. The focus of this review is the application of polysaccharides and phytochemicals in the green synthesis of gold and silver nanoparticles to afford biocomposites with novel uses in nanomedicine and as nanocomposites.

1 Introduction

The application of metallic nanoparticles is promising in many areas such as optics, biomedical sciences, drug delivery, catalysis and electronics [1–4]. Historically, the most common synthesis of metallic nanoparticles has utilised chemical reducing agents such as hydrazine, sodium citrate and sodium borohydride to reduce the corresponding precursor salts to create uniform suspensions [5]. Currently, the green synthesis of metallic nanoparticles is being investigated to improve and/or protect the environment by decreasing the use of toxic chemicals and eliminating biological risks in pharmaceutical and biomedical applications. For the development of green chemistry, Raveendran *et al.* suggested that three main factors in nanoparticle preparation should be considered: solvent choice, the use of an environmentally benign reducing agent, and the use of a non-toxic material for nanoparticle stabilisation [6]. In green nanoparticle synthesis water is commonly used as an environmentally benign solvent, replacing toxic organic solvents. Recently, biological entities have been reported as serving as both reducing and stabilising agents for green synthesis of metallic nanoparticles [7]. This review focuses on the use of polysaccharides and phytochemicals as the reducing and stabilising agents in the green synthesis of gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs).

On earth, plants synthesise $\sim 4 \times 10^{11}$ tons of carbohydrates each year, and most of these carbohydrates are polysaccharides (Fig. 1) [8]. Polysaccharides have many functionalities including hydroxyl groups and a hemiacetal reducing end that are capable of reducing precursor salts. The oxidation of polysaccharide hydroxyl groups to carbonyl groups plays an important role in the reduction of gold salts [9]. The reducing end of polysaccharides can also be used to introduce an amino functionality capable of complexing to and stabilising metallic nanoparticles [10]. Carbohydrates with such amino groups bind tightly to the surface of the AuNPs and AgNPs giving them a hydrophilic surface [11]. The use of reducing end chemistry introduces one amino group per polysaccharide chain, and provides an excellent way of measuring the exact amount of polysaccharides loaded onto a nanoparticle. This measurement can be confirmed by subjecting the particles to a heparinase I digestion and subsequent carbazole assay [11]. The application of mammalian glycosaminoglycan polysaccharides, the polysaccharide loading of gold nanoparticles prepared using reductively aminated heparin, heparin or hyaluronic acid (HA), as well as plant polysaccharides (starch, cellulose, dextran and alginic acid)

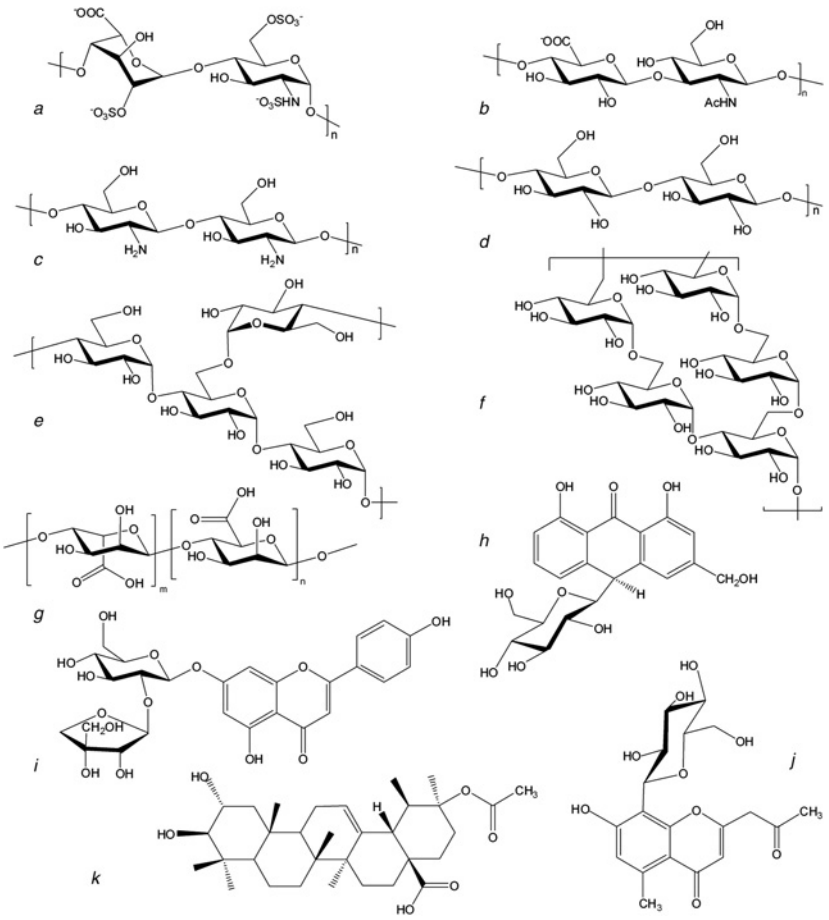

Fig. 1 *Structures of compounds reported in this review*

a Heparin
b Hyaluronic acid
c Chitosan
d Cellulose
e Starch
f Dextrose
g Alginic acid
h Aloin A
i Apiin
j Aloesin
k Guavanoic acid
a–*g* Major saccharide units of polysaccharides (adapted from [8])
h–*k* Pureplant-derived compounds

and chitosan in the synthesis of nanoparticles are addressed in this review. Currently, all of these polysaccharides are widely employed as tissue-engineering scaffolds and in drug delivery applications (as micelles, particles or hydrogels) as described in a recent review [12]. These polysaccharides are generally considered biocompatible polymers.

The second focus of this review is the synthesis of nanoparticles by phytochemicals. This field remains under-explored and is an area of research having great potential. Since ancient times, both primary and secondary metabolites of plants (phytochemicals) have demonstrated their importance in human health applications. Traditional Chinese medicine, very popular in Asian countries, actively investigates the role and biological activities of phytochemicals. For example, tea is known to contain powerful antioxidant compounds such as polyphenols [13]. The antioxidant and anticancer activities of tea is widely believed to provide important health benefits [14–17]. Tea leaf extracts have been recently used for the green synthesis of AuNPs and AgNPs [18–20]. Addressed in this review is the use of phytochemicals present in plant extracts, as pure compounds, and as present in various consumable foods to synthesise nanoparticles.

This review focuses on the introduction of two types of biologically active molecules, polysaccharides and phytochemicals, for the green synthesis of AuNPs and AgNPs. Nanoparticles prepared by biologically active polysaccharides and phytochemicals might exert synergistic effects by combining their biological activities with those of nanoparticles. The preliminary studies of the synergistic properties and potential applications of these polysaccharide and phytochemical metal nanocomposites are also reviewed.

2 Polysaccharides for gold and silver nanoparticle synthesis

2.1 Heparin

Heparin is a linear, highly acidic mammalian polysaccharide consisting of repeating, highly sulfated, 1,4-linked hexosamine and uronic acid residues. It has an average

doi: 10.1049/iet-nbt.2010.0033

molecular weight of 10^4 Da and is commonly extracted from animal tissues such as porcine intestine and bovine lung. Heparin's major disaccharide repeating unit is 2-*O*-sulfo iduronic acid 1,4-linked to *N*-sulfo, 6-*O*-sulfo glucosamine (Fig. 1*a*). It has been used as an intravenous anticoagulant drug since 1935 and has a variety of biological functions primarily resulting from its selective binding to diverse group of physiologically important proteins [21]. Biological functions include blood anticoagulation, anti-inflammation promotion of cell adhesion, cell migration and mitogenesis [21].

Heparin composites of AuNPs and AgNPs, having important biological activities, have been prepared from their respective precursor salts using 2,6-diaminopyridinyl heparin (DAPHP), which served as both a reducing and stabilising agent [22]. The reducing end of heparin was first modified with 2,6-diaminopyridine (DAP) to produce DAPHP, which is capable of tightly binding AgNPs and AuNPs. A narrow size distribution of particles was observed: 10 ± 3 nm for Au-DAPHP nanoparticles and 7 ± 3 nm for Ag-DAPHP nanoparticles, owing to the tight binding of the diaminopyridine moiety of DAPHP to the surface of the particles. Both Au-DAPHP and Ag-DAPHP nanoparticles have anticoagulant activities determined by activated partial thromboplastin time and clot dynamics in human whole blood by thromboelastography. Local anti-inflammatory activities were observed for these nanocomposites, which also did not show any significant effect on systemic hemostasis. Furthermore, Ag-DAPHP nanoparticles showed potent antimicrobial activity against *Staphylococcus aureus* (*S. aureus*) and modest activity against *Escherichia coli* (*E. coli*, Table 1). DAPHP itself had no activity against *S. aureus* or *E. coli* [23]. Both Au- and Ag-DAPHP nanoparticles exhibited anti-angiogenesis properties through inhibition of basic fibroblast growth factor-induced angiogenesis [11].

Heparin without modification of its reducing end was used for the preparation of AgNPs by thermal treatment at 70°C [24]. The concentration of heparin and the precursor salt was an important factor for the morphology and size distribution of nanoparticles. The size of the AgNPs increased as the concentration of heparin and the Ag^+ ion increased. In addition, the surface plasmon resonance (SPR) band was red-shifted with increasing concentrations of heparin and Ag^+ ion.

2.2 Hyaluronic acid

HA is a linear, high-molecular-weight, polydisperse polysaccharide widely distributed in soft connective tissues of animals. HA is comprised of 500 to several thousand repeating disaccharide units of glucuronic acid and *N*-acetyl glucosamine residues and contains no sulfo groups (Fig. 1*b*). Solutions of high-molecular-weight HA are useful in a number of medical applications owing to its biological compatibility and useful rheological properties [29]. HA-based nanoparticle composites take advantage of the biological compatibility of HA. HA was used for the formation of AuNPs and AgNPs with sizes ranging from 5 to 30 nm by thermal treatment, where HA was used as both the reducing and stabilising agent [22]. These HA nanoparticle composites have a higher particle size distribution than Au-DAPHP and Ag-DAPHP nanoparticles. Ag-HA nanoparticles exhibited potent antimicrobial activity against *S. aureus* and modest activity against *E. coli* (Table 1) [23].

2.3 Chitosan

Chitosan is a polysaccharide derived from chitin. Chitin is a major component of the exoskeleton of invertebrates such as crabs, lobsters, spiders, shrimps and insects, and the structure of chitin is similar to that of cellulose [8]. Chitin is a homopolymer composed of β(1,4)-linked *N*-acetyl-*D*-glucosamine residues (Fig. 1*c*), while cellulose consist of β(1,4)-linked D-glucose (Fig. 1*d*). Removal of *N*-acetyl groups (de-*N*-acetylation) from chitin yields chitosan, which has improved solubility, and the exposed primary amine effectively supports the immobilisation of metallic nanoparticles. Further modification of chitosan by carboxymethylation of hydroxyl and amino groups affords carboxymethyl chitosan (CMC). CMC was evaluated as a matrix material for platinum (Pt), Au and Ag nanoparticles [30]. The lack of free amines in CMC led to poor cross-linking and a limited anchoring ability, which makes CMC a poor choice as a matrix material for nanoparticles.

Chitosan-stabilised AuNPs were reported by Huang and Yang in 2004 [24]. Since then, many reports have incorporated chitosan into metallic nanoparticles for many applications (Fig. 2). The concentration of both the chitosan and precursor salt affects the morphology and size distribution of the AuNPs. The electrostatic attractive forces between the positively charged amino groups of chitosan and the negatively charged $AuCl_4^-$ ion drive the nanoparticle formation and lend the nanoparticles high stability. Sun *et al.* reported that the intrinsic viscosity of chitosan decreased during the synthesis of AuNPs, implying that some chitosan chains had degraded [31]. Increasing the Au content and temperature resulted in an increase in composite degradation.

In another report, the catalytic activity of metal-chitosan nanoparticle bioconjugates was tested for the reduction of 4-nitrophenol in the presence of $NaBH_4$ [32]. Ag-chitosan nanoparticle bioconjugates exhibited excellent catalytic activity compared with Au-chitosan nanoparticle bioconjugates. In addition, Ag-chitosan nanoparticles bioconjugates can be reused for up to seven cycles by easily separating these nanocomposites from the reaction medium. Ag-chitosan

Table 1 Observed antimicrobial properties

AgNP reductant	*S. Aureus*	*E. Coli*	*Bacillus*	*V. harveyi*	Gram+	Gram−	[Ref.]
DAPHP	potent	modest	NR[a]	NR	NR	NR	[23]
HA	potent	modest	NR	NR	NR	NR	[23]
Chitosan	NR	NR	NR	NR	potent	potent	[25]
Chitosan (film)	NR	active	active	NR	NR	NR	[26, 27]
tea-leaf extract	NR	NR	NR	active	NR	NR	[18]
Cinnamon zeylanicum bark extract	NR	active	NR	NR	NR	NR	[28]

[a]NR = not reported

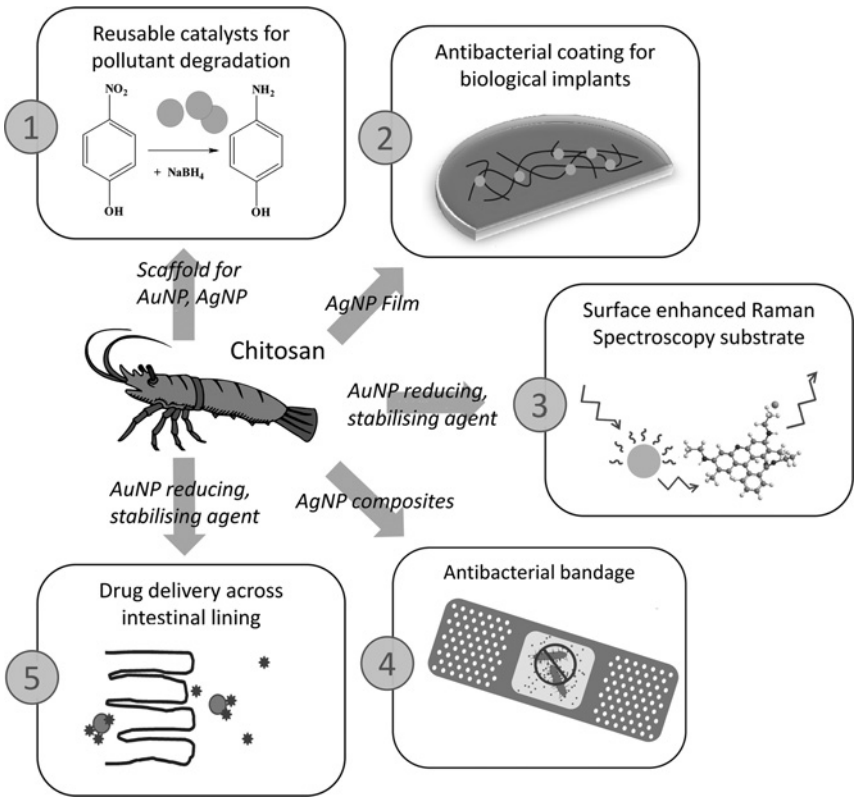

Fig. 2 *Chitosan, typically isolated from the shells of crustaceans, has been used to synthesise AuNPs and AgNPs in a wide variety of formulations*

This diverse body of functionalised nanoparticles has many potential applications. Examples taken from [24, 31, 33–35]; see text for details

nanoparticles bioconjugates also show highly potent antibacterial activity towards both Gram-positive and Gram-negative bacteria (Table 1) [25]. The same group reported earlier that chitosan enhanced nanoparticle stability, binding to AuNPs or AgNPs through the amino group [33].

The reaction temperature is also an important factor in controlling the size, shape and crystalline structure of Au-chitosan nanocomposites [34]. These composites were successfully employed as a substrate for trace analysis of amino acids by surface-enhanced Raman scattering. Interestingly, a dendritic Ag-chitosan film obtained by mixing the chitosan solution with Ag salts was also used as a surface-enhanced Raman spectroscopy substrate [35]. A similar Ag-chitosan film synthesised by a reduction of silver ions in an acidic solution of $AgNO_3$ and chitosan exhibited antibacterial activity against *E. coli* and *Bacillus* (Table 1). This film might be useful as a scaffold for wound dressings and for grafting onto various bio-implants [26]. Genipin, a cross-linking agent, was used to improve the structural reinforcement and antibacterial properties of Ag-chitosan nanoparticle bioconjugates (Table 1). AgNPs were also embedded to a genipin-crosslinked chitosan film for wound-dressing applications [27]. Another important nanomedicinal application for chitosan funcionalised AuNPs was reported by Bhumkar *et al.* [36]. Using chitosan as both a reducing and stabilising agent, the resulting Au-chitosan nanoparticles were demonstrated to improve the transport of insulin across the intestinal track of diabetic rats.

2.4 Cellulose

Cellulose is a polysaccharide consisting of a linear chain of β(1,4)-linked D-glucose (Fig. 1*d*) and is one of the most abundant polysaccharides found on earth [8]. Cellulose is a main component of plant cell walls and thus is the main component of plant fibres. Carboxymethylcellulose is an ester derivative of cellulose usually used in the food industry as a thickener and stabiliser. Cai *et al.* reported the preparation of transparent nanoporous cellulose gels obtained from an aqueous alkali hydroxide-urea solution, and these gels were immersed in precursor salt solutions ($AgNO_3$, $HAuCl_4{\cdot}3H_2O$ or $PtCl_4$) to synthesise metal nanoparticles [37]. These metal-carrying gels were dried by supercritical CO_2 providing high transmittance, porosity, surface area, moderate thermal stability and good mechanical strength. These nanomaterials have many potential applications, including their use as catalytic surfaces, use in electro-optical devices and as antibacterial surfaces.

2.5 Starch

Starch is synthesised in plants and is a mixture of α-amylose and amylopectin (Fig. 1*e*) [8]. α-Amylose is a linear polymer with α(1,4)-glycosidic linkages and amylopectin has α(1,6)-branches at every 24–30 glucose residues of the α-amylose chain [8]. Starch-stabilised and glucose-reduced AgNPs were prepared via the incubation of Ag salt with starch and glucose at 40°C for 20 h producing a particle with a mean size of 5.3 nm [6]. This AgNP solution was stable without any noticeable aggregation even after 2 months of storage. Another thermal method, an autoclaving method (15 psi, 121°C, 5 min), was introduced for the synthesis of stable AgNPs in the size range of 10–34 nm using soluble starch as both a reducing and stabilising agent [38]. These AgNPs are entrapped inside the helical amylose structure (Fig. 3), as confirmed by iodometric titration.

doi: 10.1049/iet-nbt.2010.0033

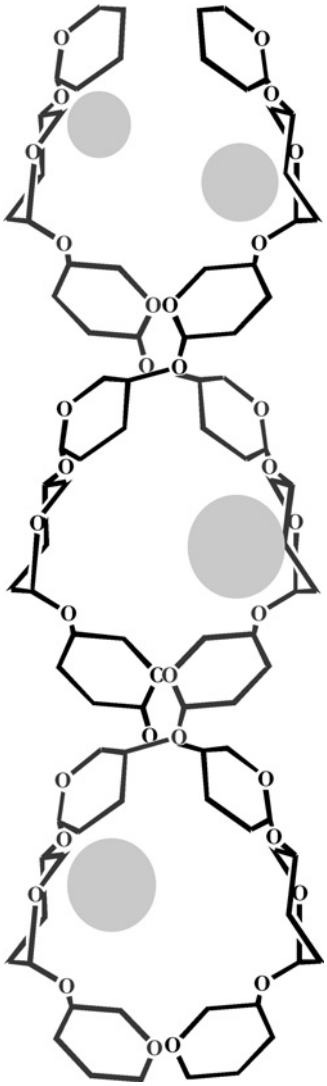

Fig. 3 *Soluble starch was used to synthesise stable AgNPs*
The nanoparticles were found to primarily reside inside helical amylose chains

The α-amylase digestion rate of Au-starch nanoparticle composites was compared with free starch, and the digestion rate was identical [39]. Interestingly, after digestion the α-amylase enzyme, and not the starch degradation products, was found to attach to the AuNPs and the enzyme catalytic activity was retained. With α-amylase bound to the nanoparticles, the nanoparticle SPR changed. Thus, monitoring the characteristic ultraviolet-visible wavelength (UV-Vis) profile of the nanoparticle SPR absorption band could be used as a direct probe for the digestion of the composite by α-amylase.

2.6 Dextran

Dextran has a complex structure and is composed of a linear chain with α(1,6)-glycosidic linkages between glucose and branches with a α(1,4)-, α(1,2)- or α(1,3)-glycosidic linkages (Fig. 1*f*) [8]. A biosensor was developed to detect concanavaline A (Con A) using aminodextran as a reducing and protecting agent [40]. The size of AuNPs and AgNPs was controlled by varying the molar ratio of the aminodextran and precursor salts. Aminodextran-protected AuNPs were used as a sensitive method to detect Con A. The aminodextran nanoparticles bind to Con A by forming a 4:1 nanoparticle:Con A complex at neutral pH. The complex can be dissociated by the disaccharide isomaltose which competitively binds to Con A. In another report, the importance of solution pH was mentioned [41]. In alkaline pH (pH $\sim$ 12) an aminodextran solution yields spherical uniform AuNPs ($\sim$20 nm) while acidic pH favours the formation of large Au crystals of other shapes. Dextran was also used as a reducing agent and surface coating material for the synthesis of stable, biocompatible AuNPs [42]. In this case, dextran was cross-linked using epichlorohydrin and aminated by ammonium hydroxide. The resulting nanoparticles exhibited colloidal stability at elevated temperatures, extreme pH, high salt concentration and in common biological buffers.

2.7 Alginic acid

Alginic acid is a polysaccharide widely distributed in the cell walls of brown algae. It has homopolymeric blocks of (1,4)-linked β-*D*-mannuronic acid (M) and α-L-glucuronic acid (G) where they are C-5 epimers of each other (Fig. 1*g*). These homopolymeric blocks are made of several types, including G-block regions, M-block regions and alternating regions [43]. Alginic acid has many applications, including use as a pharmaceutical excipient in drug products and contributions to drug delivery systems [44]. It is used in the food industry as a stabiliser and as a thickener [43]. AgNPs and AuNPs were synthesised with calcium alginate via a photochemical approach using a UV light source (365 nm wavelength) and were evaluated as a solid-phase heterogeneous catalyst for industrial applications [45]. Calcium alginate gel served as both a reducing agent and stabiliser and the resulting nanoparticles were spherical and crystalline with a size of less than 10 nm. Their catalytic activity was assessed by the reduction of 4-nitrophenol to 4-aminophenol, and was found to be very efficient.

3 Phytochemicals from plant sources in the synthesis of gold and silver nanoparticles

3.1 Plant extracts

Whole leaf extracts, which are rich in polyphenols such as flavonoids, are powerful reducing agents for the production of AuNPs and AgNPs. Therefore many publications, summarised here, have reported the use of leaves and herbs for the synthesis of nanoparticles [46–52]. Geranium (*Pelargonium graveolens*) leaf extract was used as a reducing agent for the generation of stable and crystalline AgNPs from Ag ions with an average size of 27 nm [46]. Triangular-shaped AuNPs and spherical-shaped AgNPs were synthesised using an *Aloe vera* extract [47]. Thin, flat, single-crystalline Au nanotriangles were prepared by a lemongrass extract (*Cymbopogon flexuosus*) [48]. Black tea leaf extract and Barbated Skullcup herb (*Scutellaria barbata*) extract were efficiently used to reduce Au ions to AuNPs, with applications in electrochemistry [19, 49]. Pine (*Pinus desiflora*), Persimmon (*Diopyros kaki*), Ginko (*Ginko biloba*), Magnolia (*Magnolia kobus*) and Platanus (*Platanus orientalis*) leaf extracts were successfully used to synthesise stable AgNPs with a size range of 15–500 nm from Ag ions [50]. The morphology of AuNPs made with the leaf broth of *Cinnamomum zeylanicum* was found to be dependent on the leaf broth concentration [51]. Other researchers have used the *Eclipta* leaf for the synthesis of

spherical AgNPs nanoparticles having a size of 2–6 nm [52]. Phytochemicals in tea were used to serve dual roles for the production of AuNPs: as an effective reducing agent and as a stabiliser [20]. The AuNPs generated from the tea phytochemicals exhibited remarkable in vitro stability and affinity towards prostate and breast cancer cells. These nanoparticles were non-toxic as determined by an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cell viability assay and may provide excellent applications for molecular imaging and therapy. The AgNPs synthesised by tea-leaf extracts also showed antibacterial activity against *Vibrio harveyi* (Table 1) suggesting an alternative to antibiotics in controlling *V. harveyi* infection [18]. In other work, *Cinnamon zeylanicum* bark extract and powder were compared in the synthesis of crystalline AgNPs [28]. Bark extract was more effective than powder in the production of AgNPs, suggesting a large availability of reducing agents in the bark extract. The synthesised nanoparticles showed antibacterial activity against the *E. coli* BL-21 strain (Table 1).

3.2 Whole plant extracts

Gardea-Torresdey *et al.* reported the synthesis of AuNPs and AgNPs using alfalfa (*Medicago sativa*) as a reducing agent (Fig. 4) [53–55]. The alfalfa biomass generated various shapes of AuNPs, including face-centred cubic (fcc), tetrahedral, hexagonal platelet, icosahedral multiple twinned, decahedral multiple twinned and irregular-shaped particles [53]. Icosahedral and irregular particles were formed more frequently than the rest. Interestingly, live alfalfa plants were used for the fabrication of AuNPs and AgNPs where the agar was used as a reducing agent and the plant bio-synthesised the nanoparticles [54, 55]. Alfalfa plants were grown in an $AuCl_4$- or $AgNO_3$-rich environment and the formation of crystalline Au or Ag metal inside the living plants was confirmed by X-ray absorption studies and transmission electron microscopy (TEM). In a separate study using alfalfa as the reducing agent, the control of AgNP size was based on different pH conditions as reported by Tavera-Davila *et al.* [56]. A cubic-like structure was obtained with the optimum pH 10 with an average particle size of 4.09 nm.

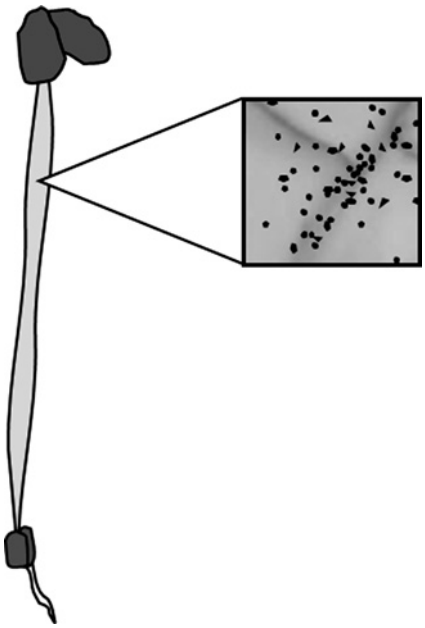

Fig. 4 *AuNPs and AgNPs were synthesised inside living alfalfa (Medicago sativa) plants*

The precursor salts were reduced by the agar growth media used; once transported into the plants, the reduced metals were deposited into nanoparticles of various shapes and sizes

Three categories of whole plant extracts (xerophytes-*Bryophyllum* sp., mesophytes-*Cyprus* sp. and hydrophytes-*Hydrilla* sp.) which grow under extreme conditions were also successfully used to synthesise AgNPs having fcc structures with 2–5 nm diameter [57]. The authors proposed a detailed mechanism explaining how the reduction of Ag ions with these plants could be owing to different metabolites (organic acids/quinines), metabolic fluxes and other oxido-reductively labile metabolites (ascorbates/catechol/protocatacheuic acid). Seeds and gum arabic of cumin (*Cuminum cyminum*) were used for the synthesis of spherical AuNPs, which were stable over a period of 4 weeks [58]. The AuNP size was in the range of 10–15 nm, which the phyochemical coating expanded to a hydrodynamic radius of 77 ± 1 nm by dynamic light scattering. A zeta potential of -15 ± 1 mV indicates that there was no tendency of the particles to aggregate. In addition, excellent in vitro stability was observed in a series of solutions, including 0.5% cysteine, 0.2 M histidine, 0.5% human serum albumin, 0.5% bovine serum albumin, 5% NaCl and phosphate buffers (pH 5, 7 and 9). A fibroblast cell-based MTT assay showed that these particles were non-cytotoxic and may provide a new scaffold in the area of molecular imaging and therapy.

While the focus of this review is phytochemicals, microorganisms have also been used to synthesise AuNPs and AgNPs. The fungus *Fusarium oxysporum* has been used to synthesise both AuNP [59] and AgNPs [60]. The crystalline AgNPs were found to be 5–15 nm in dimension [60]. The precursor salts were added to the cellular broth, reducing the salts extracellularly and creating extremely stable nanoparticles in solution. The authors propose that the precursor salts are reduced via enzymes secreted by the fungus, and are further stabilised by complexing with secreted fungal proteins [60]. Further analysis of proteins isolated from the fungal broth indicated that the reduction of the precursor salts was owing to the presence of NADH-dependent reductases [59]. The resulting nanoparticles were found to be stable, showing no signs of aggregation, even after 1 month [60].

Elsewhere, Lengke *et al.* report the use of cyanobacteria *Plectonema boryanum* to synthesise AgNPs in solution [61]. The silver ions were reduced both intra and extracellularly, and the reduction mechanisms are hypothesised to involve metabolic processes and also cellular extracts from dead cyanobacteria. The resulting spherical AgNPs and Ag platelets were of relatively small size within the cell (<10 nm) but were considerably larger and more varied in composition outside of the cell (1–200 nm). Intracellular synthesis of much larger AgNPs (up to 200 nm) was also reported in *Pseudomonas stutzeri* [62]. These nanoparticles were found to be crystalline in nature and exhibit a wide variety of shapes, including triangles and hexagons. Compositions of the crystals were also found to vary, including crystals composed of Ag_2S. The ability of microbes reduce silver and synthesise AgNPs was explored using *Morganella* sp. bacteria [63]. The spherical nanoparticles were found to be crystalline and $\sim$20 nm in dimension, and were observed to be highly stable over a 6-month period. The ability of *Morganella* sp. to form AgNPs is believed to be related to the bacteria's tolerance

doi: 10.1049/iet-nbt.2010.0033

of high silver environments, and the authors believe the presence of silver-specific proteins which are part of the bacteria's silver resistance are key to the formation of the AgNPs. The use of bacteria and fungi to form AuNPs and AgNPs is of interest in the development of new thin film and surface coating technologies, possibly as a means of developing the ability to synthesise metallic nanoparticles directly on the surface of biochemically tailored organic matrices.

3.3 Pure compounds from plants

In addition to whole plant extracts, pure compounds isolated from plants are being utilised for metallic nanoparticle synthesis. Two active glycosides from Cape aloe (*Aloe ferox* Miller), aloin A and aloesin (Figs. 1*h* and *j*), were used as stabilisers in the preparation of AuNPs and AgNPs [64]. The size and shape of the nanoparticles differed by varying reaction conditions such as temperature, reaction time and reducing agents (Fig. 5). In another experiment, the cellular uptake by macrophages and HeLa cells was investigated using aloin A-stabilised and aloesin-stabilised AuNPs (50 nm). Apiin (apigenin-7-*O*-apioglucoside, Fig. 1*i*), which is a flavonoid glycoside that is abundant in parsley and celery, was successfully isolated from henna leaves (*Lawsonia inermis*) and used for the synthesis of anisotropic AuNPs and quasi-spherical AgNPs with an average size of 21–30 nm [65]. By using fourier transform infrared (FT-IR) spectroscopy, they confirmed that the carbonyl group of apiin contributes to the interaction between the nanoparticles and apiin. Guavanoic acid (Fig. 1*k*), a triterpenoid from apple guava (*Psidium guajava*), generated AuNPs with protein tyrosine phosphatase 1B inhibitory activity that had in vitro stability in various physiological medium including 10% saline, 0.2 M histidine, 0.2 M cysteine, 0.5% bovine serum albumin, 0.5% human serum albumin and phosphate buffers (pH 5, 7, and 9) [66].

3.4 Food sources from plant origin

Food sources from plant origin have also been used for the synthesis of metallic nanoparticles. Shukla *et al.* demonstrated the use of soybean (*Glycine max*) extract for the production and stabilisation of biocompatible AuNPs [67]. Soybeans are composed of water-soluble proteins (41%; globulins and albumins), carbohydrates (28%; sucrose, raffinose and stachyose), fat and dietary fibre (16%), saponins (2%), isoflavons (1%) and amino acids (1%) [67]. The anti-oxidant phytochemicals in soybean extracts were found to play an important role in the reduction of Au salts and stabilisation of the resulting nanoparticles. AuNPs exhibited in vitro stability in various buffers and were non-toxic as determined by an MTT cell viability assay. Soybean leaf extract was also used for the synthesis of AgNPs with a size range of 25–100 nm [68]. Honey, made from flower nectar, was used as the reducing and capping agent for AuNPs, producing either anisotropic or spherical nanocrystals [69]. FT-IR measurements indicated that the surface-bound stabilising agent is a protein, bound to the Au surface through amine groups rather than carboxylic groups. Next, the same author reported the use of edible mushroom (*Volvariella volvacea*) extract for the biosynthesis of AuNPs, AgNPs and Au-AgNPs, which were found to be photoluminescent and

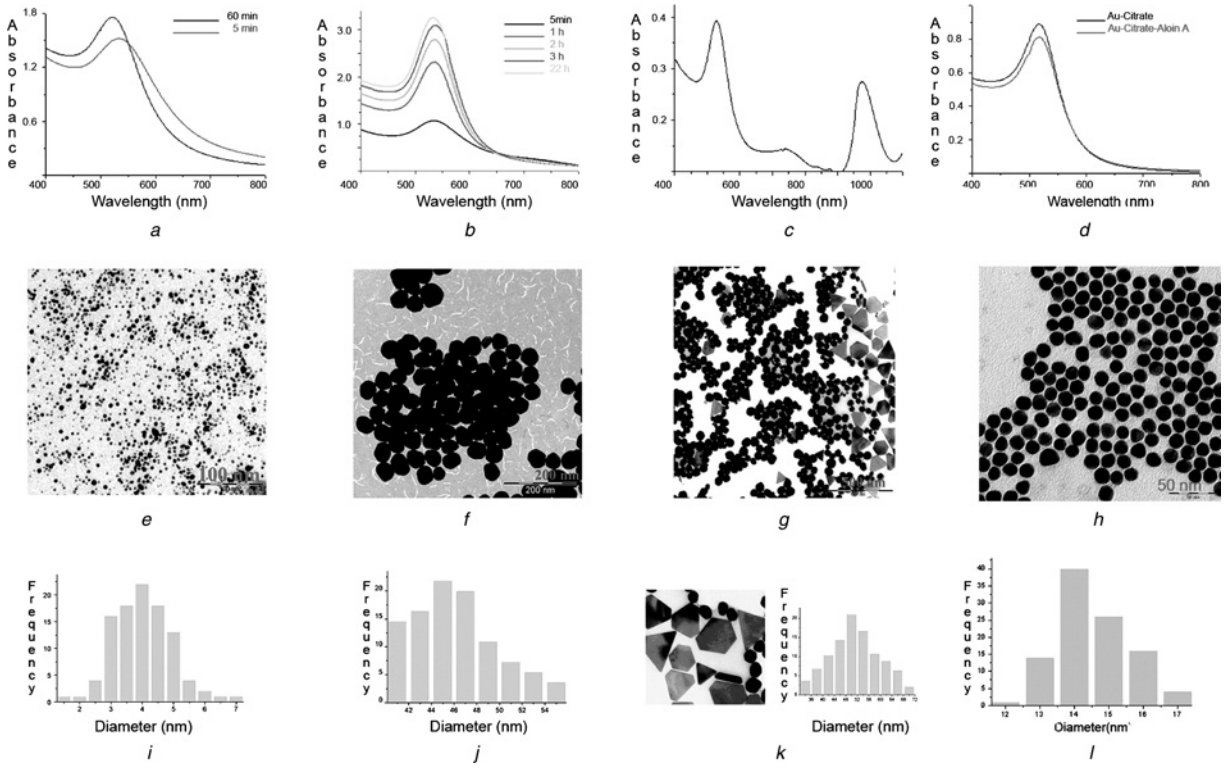

Fig. 5 *Characterisation data for various Au nanoparticles synthesised using aloesin or aloin and different reducing agents (taken from [64])*

Shown are the UV-Vis spectra, TEM micrographs and corresponding particle size distribution charts of each particle type
a, *e*, *i* Au-aloesin reduced by 0.1 M $NaBH_4$, 25°C
b, *f*, *j* Au-aloesin reduced by 0.01 M $NaBH_4$, 55°C
c, *g*, *k* Au-aloin A reduced by 1 M citric acid, 25°C
d, *h*, *l* Au-aloin A prepared by a ligand exchange reaction

highly crystalline [69, 70]. Proteins were bound to AuNPs and AgNPs through free amino groups and carboxylic groups of the amino acid residues, respectively. Oat (*Avena sativa*) biomass has also been used for AuNP formation with tetrahedral, decahedral, hexagonal, icosahedral multitwinned and irregular shapes in which the pH of the reaction influenced the size of particles [71]. At pH values of 3–4, smaller nanoparticles were observed, whereas at pH 2, larger nanoparticles were observed. AuNPs were synthesised using oat and wheat (*Triticum* sp.) biomass in aqueous solutions under acidic conditions (pH 4) [72]. The resulting nanoparticles were then extracted with sodium citrate or cetyltrimethylammonium bromide (capping agents which coat the nanoparticle) allowing the nanoparticles to be isolated from the biomass and analysed. Using pear fruit extract (*Pryus* sp.), triangular and hexagonal nanoplates of AuNPs were synthesised under alkaline conditions [73]. The edge lengths of these nanoplates ranged from 200 to 500 nm without any substantial impurities. Biomass of the edible brown alga *Fucus vesiculosus* (also called bladder wrack) reduced Au(III) to Au(0) within an optimum pH range of 4–9 [9]. Hydroxyl groups in algal polysaccharides are abundant and contribute to gold reduction. Researchers suggested that the reduction of Au(III) to Au(0) occurs through the oxidation of hydroxyl groups to carbonyl groups as shown below [9]

$$AuCl_4^- + 3R - OH \rightarrow Au^0 + 3R = O + 3H^+ + 4Cl^-$$

4 Conclusions

A growing need for sustainability initiatives in the field of nanotechnology has brought about the development of green synthetic procedures for the creation of AuNPs and AgNPs, a development which is rapidly replacing traditional chemical syntheses. This transition has many advantages, including the decreased use of chemicals that are toxic to our health and environment and the creation of a collection of nanocomposites with many novel applications in nanobiotechnology. Recently, various types of natural products which serve a dual role as both the reducing and stabilising agents have been used in the synthesis of bioactive nanoparticles, including the polysaccharides and phytochemicals discussed here.

As summarised in this review, polysaccharides and phytochemicals possess their own biological activities which together with the intrinsic biological activities of metallic nanoparticles often show synergistic effects as nanocomposites, such as HA-based nanoparticles. By itself, the bioactive polysaccharide HA exhibits excellent biocompatibility, giving it several medical and cosmetic applications including its use as a biological scaffold for the treatment and healing of wounds. Silver, silver ions, and more recently AgNPs, are known to possess antibacterial and antimicrobial properties. Together, AgNPs nanoparticles synthesised by HA possess a synergetic antibacterial activity and biocompatibility that makes them a good candidate for use in medical applications. One day these Ag-HA nanocomposites may well become a common place part of medical devices, integrated into everything from surgical tools to the bandages we use for minor cuts and scrapes. As the field of nanobiotechnology grows, our use and development of biologically active natural products in composite nanoparticles will continue to expand. Fortunately, nature has provided a massive number of natural products, many of which will be good candidates for use in green nanoparticle syntheses as outlined here.

5 Acknowledgments

This work was supported by the Basic Science Research Program of the National Research Foundation of Korea (NRF), funded by the Ministry of Education, Science and Technology (grant number: 2010–0004730) to Y.P. and by the National Institutes of Health of the U.S. Department of Health and Human Services (grant number: GM090257) to R.L.

6 References

1 Sperling, R.A., Parak, W.J.: 'Surface modification, functionalization and bioconjugation of colloidal inorganic nanoparticles', *Philos. Trans. A, Math. Phys. Eng. Sci.*, 2010, **368**, (1915), pp. 1333–1383
2 Ghosh, P., Han, G., De, M., Kim, C.K., Rotello, V.M.: 'Gold nanoparticles in delivery applications', *Adv. Drug Deliv. Rev.*, 2008, **60**, (11), pp. 1307–1315
3 Hutchings, G.J., Brust, M., Schmidbaur, H.: 'Gold-an introductory perspective', *Chem. Soc. Rev.*, 2008, **37**, (9), pp. 1759–1765
4 Giljohann, D.A., Seferos, D.S., Daniel, W.L., Massich, M.D., Patel, P.C., Mirkin, C.A.: 'Gold nanoparticles for biology and medicine', *Angew. Chem., Int. Ed. Engl.*, 2010, **49**, (19), pp. 3280–3294
5 Zhou, J., Ralston, J., Sedev, R., Beattie, D.A.: 'Functionalized gold nanoparticles: synthesis, structure and colloid stability', *J. Colloid Interface Sci.*, 2009, **331**, (2), pp. 251–262
6 Raveendran, P., Fu, J., Wallen, S.L.: 'Completely "green" synthesis and stabilization of metal nanoparticles', *J. Am. Chem. Soc.*, 2003, **125**, (46), pp. 13940–13941
7 Thakkar, K.N., Mhatre, S.S., Parikh, R.Y.: 'Biological synthesis of metallic nanoparticles', *Nanomedicine*, 2010, **6**, (2), pp. 257–262
8 Lindhorst, T.K.: 'Structure of saccharides, essentials of carbohydrate chemistry and biochemistry' (Wiley-VCH, 2007, 3rd edn.)
9 Mata, Y.N., Torres, E., Blazquez, M.L., Ballester, A., Gonzalez, F., Munoz, J.A.: 'Gold(III) biosorption and bioreduction with the brown alga Fucus vesiculosus', *J. Hazard Mater.*, 2009, **166**, (2–3), pp. 612–618
10 Nadkarni, V.D., Pervin, A., Linhardt, R.J.: 'Directional immobilization of heparin onto beaded supports', *Anal. Biochem.*, 1994, **222**, (1), pp. 59–67
11 Kemp, M.M., Kumar, A., Mousa, S., *et al.*: 'Gold and silver nanoparticles conjugated with heparin derivative possess anti-angiogenesis properties', *Nanotechnology*, 2009, **20**, (45), article id. 455104 (7pp)
12 Baldwin, A.D., Kiick, K.L.: 'Polysaccharide-modified synthetic polymeric biomaterials', *Biopolymers*, 2010, **94**, (1), pp. 128–140
13 Wang, Y., Ho, C.T.: 'Polyphenolic chemistry of tea and coffee: a century of progress', *J. Agric. Food Chem.*, 2009, **57**, (18), pp. 8109–8114
14 Chen, D., Dou, Q.P.: 'Tea polyphenols and their roles in cancer prevention and chemotherapy', *Int. J. Mol. Sci.*, 2008, **9**, (7), pp. 1196–1206
15 Khan, N., Mukhtar, H.: 'Multitargeted therapy of cancer by green tea polyphenols', *Cancer Lett.*, 2008, **269**, (2), pp. 269–280
16 Yang, C.S., Wang, X., Lu, G., Picinich, S.C.: 'Cancer prevention by tea: animal studies, molecular mechanisms and human relevance', *Nat. Rev. Cancer*, 2009 , **9**, pp. 429–439
17 Lamoral-Theys, D., Pottier, L., Dufrasne, F., *et al.*: 'Natural polyphenols that display anticancer properties through inhibition of kinase activity', *Curr. Med. Chem.*, 2010, **17**, (9), pp. 812–825
18 Vaseeharan, B., Ramasamy, P., Chen, J.C.: 'Antibacterial activity of silver nanoparticles (AgNps) synthesized by tea leaf extracts against pathogenic Vibrio harveyi and its protective efficacy on juvenile Feneropenaeus indicus', *Lett. Appl. Microbiol.*, 2010, **50**, (4), pp. 352–356
19 Begum, N.A., Mondal, S., Basu, S., Laskar, R.A., Mandal, D.: 'Biogenic synthesis of Au and Ag nanoparticles using aqueous solutions of Black Tea leaf extracts', *Colloids Surf. B Biointerfaces*, 2009, **71**, (1), pp. 113–118
20 Nune, S.K., Chanda, N., Shukla, R., *et al.*: 'Green nanotechnology from tea: phytochemicals in tea as building blocks for production of biocompatible gold nanoparticles', *J. Mater. Chem.*, 2009, **19**, (19), pp. 2912–2920

doi: 10.1049/iet-nbt.2010.0033

21 Laremore, T.N., Zhang, F., Dordick, J.S., Liu, J., Linhardt, R.J.: 'Recent progress and applications in glycosaminoglycan and heparin research', *Curr. Opin. Chem. Biol.*, 2009, **13**, (5–6), pp. 633–640

22 Kemp, M.M., Kumar, A., Mousa, S., *et al.*: 'Synthesis of gold and silver nanoparticles stabilized with glycosaminoglycans having distinctive biological activities', *Biomacromolecules*, 2009, **10**, (3), pp. 589–595

23 Kemp, M.M., Kumar, A., Clement, D., Ajayan, P., Mousa, S., Linhardt, R.J.: 'Hyaluronan- and heparin-reduced silver nanoparticles with antimicrobial properties', *Nanomedicine (Lond)*, 2009, **4**, (4), pp. 421–429

24 Wei, D., Sun, W., Qian, W., Ye, Y., Ma, X.: 'The synthesis of chitosan-based silver nanoparticles and their antibacterial activity', *Carbohydr. Res.*, 2009, **344**, (17), pp. 2375–2382

25 Thomas, V., Yallapu, M.M., Sreedhar, B., Bajpai, S.K.: 'Fabrication, characterization of chitosan/nanosilver film and its potential antibacterial application', *J. Biomater. Sci. Polym. Ed.*, 2009, **20**, (14), pp. 2129–2144

26 Liu, B.S., Huang, T.B.: 'Nanocomposites of genipin-crosslinked chitosan/silver nanoparticles – structural reinforcement and antimicrobial properties', *Macromol. Biosci.*, 2008, **8**, (10), pp. 932–941

27 Sathishkumar, M., Sneha, K., Won, S.W., Cho, C.W., Kim, S., Yun, Y.S.: 'Cinnamon zeylanicum bark extract and powder mediated green synthesis of nano-crystalline silver particles and its bactericidal activity', *Colloids Surf. B Biointerfaces*, 2009, **73**, (2), pp. 332–338

28 Huang, H., Yang, X.: 'Synthesis of polysaccharide-stabilized gold and silver nanoparticles: a green method', *Carbohydr. Res.*, 2004, **339**, (15), pp. 2627–2631

29 Doughty, M.J., Glavin, S.: 'Efficacy of different dry eye treatments with artificial tears or ocular lubricants: a systematic review', *Ophthalmic Physiol. Opt.*, 2009, **29**, (6), pp. 573–583

30 Laudenslager, M.J., Schiffman, J.D., Schauer, C.L.: 'Carboxymethyl chitosan as a matrix material for platinum, gold, and silver nanoparticles', *Biomacromolecules*, 2008, **9**, (10), pp. 2682–2685

31 Sun, C., Qu, R., Chen, H., *et al.*: 'Degradation behavior of chitosan chains in the 'green' synthesis of gold nanoparticles', *Carbohydr. Res.*, 2008, **343**, (15), pp. 2595–2599

32 Wei, D., Ye, Y., Jia, X., Yuan, C., Qian, W.: 'Chitosan as an active support for assembly of metal nanoparticles and application of the resultant bioconjugates in catalysis', *Carbohydr. Res.*, 2010, **345**, (1), pp. 74–81

33 Wei, D., Qian, W.: 'Facile synthesis of Ag and Au nanoparticles utilizing chitosan as a mediator agent', *Colloids Surf. B Biointerfaces*, 2008, **62**, (1), pp. 136–142

34 Potara, M., Maniu, D., Astilean, S.: 'The synthesis of biocompatible and SERS-active gold nanoparticles using chitosan', *Nanotechnology*, 2009, **20**, (31), article id. 315602 (7pp)

35 Wei, D., Qian, W., Wu, D., Xia, Y., Liu, X.: 'Synthesis, properties, and surface enhanced Raman scattering of gold and silver nanoparticles in chitosan matrix', *J. Nanosci. Nanotechnol.*, 2009, **9**, (4), pp. 2566–2573

36 Bhumkar, D.R., Joshi, H.M., Sastry, M., Pokharkar, V.B.: 'Chitosan reduced gold nanoparticles as novel carriers for transmucosal delivery of insulin', *Pharm. Res.*, 2007, **24**, (8), pp. 1415–1426

37 Cai, J., Kimura, S., Wada, M., Kuga, S.: 'Nanoporous cellulose as metal nanoparticles support', *Biomacromolecules*, 2009, **10**, (1), pp. 87–94

38 Vigneshwaran, N., Nachane, R.P., Balasubramanya, R.H., Varadarajan, P.V.: 'A novel one-pot "green" synthesis of stable silver nanoparticles using soluble starch', *Carbohydr. Res.*, 2006, **341**, (12), pp. 2012–2018

39 Deka, J., Paul, A., Ramesh, A., Chattopadhyay, A.: 'Probing Au nanoparticle uptake by enzyme following the digestion of a starch-Au-nanoparticle composite', *Langmuir*, 2008, **24**, (18), pp. 9945–9951

40 Ma, Y., Li, N., Yang, C., Yang, X.: 'One-step synthesis of amino-dextran-protected gold and silver nanoparticles and its application in biosensors', *Anal. Bioanal. Chem.*, 2005, **382**, (4), pp. 1044–1048

41 Morrow, B.J., Matijevic, E., Goia, D.V.: 'Preparation and stabilization of monodisperse colloidal gold by reduction with aminodextran', *J. Colloid Interface Sci.*, 2009, **335**, (1), pp. 62–69

42 Jang, H., Kim, Y.K., Ryoo, S.R., Kim, M.H., Min, D.H.: 'Facile synthesis of robust and biocompatible gold nanoparticles', *Chem. Commun. (Camb.)*, 2010, **46**, (4), pp. 583–585

43 George, M., Abraham, T.E.: 'Polyionic hydrocolloids for the intestinal delivery of protein drugs: alginate and chitosan – a review', *J. Control Release*, 2006, **114**, (1), pp. 1–14

44 Tonnesen, H.H., Karlsen, J.: 'Alginate in drug delivery systems', *Drug Dev. Ind. Pharm.*, 2002, **28**, (6), pp. 621–630

45 Saha, S., Pal, A., Kundu, S., Basu, S., Pal, T.: 'Photochemical green synthesis of calcium-alginate-stabilized Ag and Au nanoparticles and their catalytic application to 4-nitrophenol reduction', *Langmuir*, 2010, **26**, (4), pp. 2885–2893

46 Shankar, S.S., Ahmad, A., Sastry, M.: 'Geranium leaf assisted biosynthesis of silver nanoparticles', *Biotechnol. Prog.*, 2003, **19**, (6), pp. 1627–1631

47 Chandran, S.P., Chaudhary, M., Pasricha, R., Ahmad, A., Sastry, M.: 'Synthesis of gold nanotriangles and silver nanoparticles using Aloe vera plant extract', *Biotechnol. Prog.*, 2006, **22**, (2), pp. 577–583

48 Shankar, S.S., Rai, A., Ankamwar, B., Singh, A., Ahmad, A., Sastry, M.: 'Biological synthesis of triangular gold nanoprisms', *Nat. Mater.*, 2004, **3**, (7), pp. 482–488

49 Wang, Y., He, X., Wang, K., Zhang, X., Tan, W.: 'Barbated Skullcup herb extract-mediated biosynthesis of gold nanoparticles and its primary application in electrochemistry', *Colloids Surf. B Biointerfaces*, 2009, **73**, (1), pp. 75–79

50 Song, J.Y., Kim, B.S.: 'Rapid biological synthesis of silver nanoparticles using plant leaf extracts', *Bioprocess Biosyst. Eng.*, 2009, **32**, (1), pp. 79–84

51 Smitha, S.L., Philip, D., Gopchandran, K.G.: 'Green synthesis of gold nanoparticles using Cinnamomum zeylanicum leaf broth', *Spectrochim. Acta A Mol. Biomol. Spectrosc.*, 2009, **74**, (3), pp. 735–739

52 Jha, A.K., Prasad, K., Kumar, V.: 'Biosynthesis of silver nanoparticles using Eclipta leaf', *Biotechnol. Prog.*, 2009, **25**, (5), pp. 1476–1479

53 Gardea-Torresdey, J.L., Tiemann, K.J., Gamez, G., Dokken, K., Tehuacanero, S., José-Yacamán, M.: 'Gold nanoparticles obtained by bio-precipitation from gold(III) solutions', *J. Nanopart Res.*, 1999, **1**, (3), pp. 397–404

54 Gardea-Torresdey, J.L., Parsons, J.G., Gomez, E., *et al.*: 'Formation and growth of Au nanoparticles inside live alfalfa plants', *Nano Lett.*, 2002, **2**, (4), pp. 397–401

55 Gardea-Torresdey, J.L., Gomez, E., Peralta-Videa, J.R., Parsons, J.G., Troiani, H., Jose-Yacaman, M.: 'Alfalfa sprouts: a natural source for the synthesis of silver nanoparticles', *Langmuir*, 2003, **19**, (4), pp. 1357–1361

56 Tavera-Davila, L., Liu, H.B., Herrera-Becerra, R., Canizal, G., Balcazar, M., Ascencio, J.A.: 'Analysis of Ag nanoparticles synthesized by bioreduction', *J. Nanosci. Nanotechnol.*, 2009, **9**, (3), pp. 1785–1791

57 Jha, A.K., Prasad, K., Kulkarni, A.R.: 'Plant system: nature's nanofactory', *Colloids Surf. B Biointerfaces*, 2009, **73**, (2), pp. 219–223

58 Katti, K., Chanda, N., Shukla, R., *et al.*: 'Green nanotechnology from cumin phytochemicals: generation of biocompatible gold nanoparticles', *Int. J. Green Nanotechnol. Biomed.*, 2009, **1**, (1), B39–B52

59 Mukherjee, P., Senapati, S., Mandal, D., *et al.*: 'Extracellular synthesis of gold nanoparticles by the fungus *Fusarium Oxysporum*', *ChemBioChem*, 2009, **3**, (5), pp. 461–463

60 Ahmad, A., Mukherjee, P., Senapati, S., *et al.*: 'Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium Oxysporum*', *Colloids Surf. B Biointerfaces*, 2003, **28**, (4), pp. 313–318

61 Lengke, M.F., Fleet, M.E., Southam, G.: 'Biosynthesis of silver nanoparticles by filamentous cyanobacteria from a silver (I) nitrate complex', *Langmuir*, 2007, **23**, (5), pp. 2694–2699

62 Klaus, T., Joerger, R., Olsson, E., Granqvist, C.: 'Silver-based crystalline nanoparticles, microbially fabricated', *Proc. Natl. Acad. Sci. USA*, 1999, **96**, (24), pp. 13611–13614

63 Parikh, R.Y., Singh, S., Prasad, L.V., Patole, M.S., Sastry, M., Shouche, Y.: 'Extracellular synthesis of crystalline silver nanoparticles and molecular evidence of silver resistance from *Morganella* sp.: towards understanding biochemical synthesis mechanism', *ChemBioChem*, 2008, **9**, (9), pp. 1415–1422

64 Krpetic, Z., Scari, G., Caneva, E., Speranza, G., Porta, F.: 'Gold nanoparticles prepared using cape aloe active components', *Langmuir*, 2009, **25**, (13), pp. 7217–7221

65 Kasthuri, J., Veerapandian, S., Rajendiran, N.: 'Biological synthesis of silver and gold nanoparticles using apiin as reducing agent', *Colloids Surf. B Biointerfaces*, 2009, **68**, (1), pp. 55–60

66 Khaleel Basha, S., Govindaraju, K., Manikandan, R., Ahn, J.S., Bae, E.Y., Singaravelu, G.: 'Phytochemical mediated gold nanoparticles and their PTP 1B inhibitory activity', *Colloids Surf. B Biointerfaces*, 2010, **75**, (2), pp. 405–409

67 Shukla, R., Nune, S.K., Chanda, N., *et al.*: 'Soybeans as a phytochemical reservoir for the production and stabilization of biocompatible gold nanoparticles', *Small*, 2008, **4**, (9), pp. 1425–1436

68 Vivekanandhan, S., Misra, M., Mohanty, A.K.: 'Biological synthesis of silver nanoparticles using Glycine max (soybean) leaf extract: an investigation on different soybean varieties', *J. Nanosci. Nanotechnol.*, 2009, **9**, (12), pp. 6828–6833

69 Philip, D.: 'Honey mediated green synthesis of gold nanoparticles', *Spectrochim. Acta A Mol. Biomol. Spectrosc.*, 2009, **73**, (4), pp. 650–653

70 Philip, D.: 'Biosynthesis of Au, Ag and Au–Ag nanoparticles using edible mushroom extract', *Spectrochim. Acta A Mol. Biomol. Spectrosc.*, 2009, **73**, (2), pp. 374–381
71 Armendariz, V., Herrera, I., Peralta-Videa, J.R., *et al.*: 'Size controlled gold nanoparticle formation by Avena sativa biomass: use of plants in nanobiotechnology', *J. Nanopart Res.*, 2004, **6**, (4), pp. 377–382
72 Armendariz, V., Parsons, J.G., Lopez, M.L., Peralta-Videa, J.R., Jose-Yacaman, M., Gardea-Torresdey, J.L.: 'The extraction of gold nanoparticles from oat and wheat biomasses using sodium citrate and cetyltrimethylammonium bromide, studied by x-ray absorption spectroscopy, high-resolution transmission electron microscopy, and UV-visible spectroscopy', *Nanotechnology*, 2009, **20**, (10), article id. 105607 (8pp)
73 Ghodake, G.S., Deshpande, N.G., Lee, Y.P., Jin, E.S.: 'Pear fruit extract-assisted room-temperature biosynthesis of gold nanoplates', *Colloids Surf. B Biointerfaces*, 2010, **75**, (2), pp. 584–589

doi: 10.1049/iet-nbt.2010.0033