Research Article

Molecular Recognition and Transport of Active Pharmaceutical Ingredients on Anionic Calix[4]arene-Capped Silver Nanoparticles

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A series of six anionic calix[4]arenes, having sulphonate, carboxylate, or phosphonate functions at either the para-aromatic position or the phenolic face were used to cap silver nanoparticles. Their molecular recognition properties were studied with regard to three active pharmaceutical ingredients, chlorhexidine, chloramphenicol, and gentamycin sulfate. Of these APIs chlorhexidine is known to form cocrystals with the anionic calix[4]arenes, gentamicin sulfate is an aminoglycosidic antibiotic, and chloramphenicol is a neutral antibiotic. As expected the former two APIs show clear complexation behavior as demonstrated by shifts in the visible spectra whereas the last shows no modification in the wavelength of the plasmon resonance of the silver nanoparticles.

1. Introduction

The noble metal nanoparticles are formed by the reduction of a suitable metal salt, for example, chloroauric acid or silver nitrate to the zero oxidation state in the presence of a suitable capping molecule or polymer to stabilize them [1]. They are well known for their ability to act as sensors for molecular interactions by means of shifts in the plasmon resonance absorption [2]. A great amount of work exists concerning their biological properties [3], including biological imaging [4], of particular interest are the antibacterial effects of both the silver and gold nanoparticles [5, 6]. Indeed the silver nanoparticles have seen commercial usage in areas ranging from hygiene (disinfection of socks) [7] to treatment of computer keyboards [8].

The calix[n]arenes are a group of supramolecular systems [9] widely studied for their ability to complex a very wide

range of molecules and ions [10]. The relative ease with which the calix[n]arenes can be modified at either aromatic para-position or at the phenolic face, [11] has made them perhaps the most attractive of organic hosts. Interest in the biochemistry of the calix[n]arenes has grown dramatically with emergence of a wide range of water soluble derivatives [12]. Their direct behavior to act as Active Pharmaceutical Ingredients (APIs) is now emerging, [13] with activities ranging from anti-viral, [14] anti-bacterial, [15] enzyme activators [16] or blockers [17] anti-coagulant, [18] through anti-cancer [19] to detoxification [20]. As with other supramolecular systems the calix[n]arenes provide means to transport APIs in the solid-state as co-crystals, [21], as solubilizing agents, [22], or as colloidal suspensions, via solid lipid nanoparticle formation [23] or via the formation of cocolloidal complexes with highly hydrophobic partners [24]. Their possible pharmaceutical applications have been made more attractive by a lack of

SCHEME 1: Structures of the calix[n]arene studied.

SCHEME 2: Structures of the APIs evaluated: (a) chlorhexidine, (b) chloramphenicol, and (c) gentamycin sulfate.

hemolytic effects [25], an absence of an immune response [26], and a clear lack of in vivo toxicity [27].

The combination of the useful biopharmaceutical properties of the calix[n]arenes with known biological properties thus makes them attractive as biosensors [28] or as transporters or more interestingly by using the calix[n]arenes to act capping stabilisers for nanoparticles and the using their complexation capacities towards APIs to form multifunction APU cocktails.

2. Experimental

2.1. Synthesis. *para*-Sulfonatocalix[4]arene, **1**_{SO₃H}, was synthesized as per the literature method and physical characteristics correspond to the literature values [27]. The two

TABLE 1: Maximum wavelength of each Ag_NP_Calix[n]arene as a matter of time of preparation.

Compounds	λ after 1 hour mixing	λ after 24 hours mixing
Ag_NP_1 _{SO₃H}	400 nm	390 nm
Ag_NP_2 _{SO₃H}	390 nm	390 nm
Ag_NP_3 _{SO₃H}	390 nm	380 nm
Ag_NP_4 _{COOH}	410 nm	420 nm
Ag_NP_5 _{COOH}	390 nm	400 nm
Ag_NP_6 _{PO₃H₂}	390 nm	400 nm

3-sulfonatopropoxy derivatives, **2**_{SO₃H} and **3**_{SO₃H}, were prepared by the method of Hwang et al. [29] or Shinkai et al. [30] and the physical properties are in accord with the literature values. *tert*-Butyltetra carboxymethoxy calix[4]arene **4**_{COOH}

FIGURE 1: The UV visible spectra of Ag_NP_Calix[n]arene after (a) 1 hour and after (b) 24 hours.

TABLE 2: The slopes of the variation of the maximum wavelength of Ag_NP_Calix[n]arene against the concentration of chlorhexidine.

Compounds	Slope (nm/M)	Rank
After 1 hour mixing with chlorhexidine		
Ag_NP_3SO ₃ H	1.1×10^6	1
Ag_NP_6PO ₃ H ₂	4×10^5	2
Ag_NP_1SO ₃ H	3.6×10^5	3
Ag_NP_2SO ₃ H	2×10^5	4
Ag_NP_5COOH	2×10^5	4
Ag_NP_4COOH	0	5
After 24 hours mixing with chlorhexidine		
Ag_NP_2SO ₃ H	1×10^6	1
Ag_NP_1SO ₃ H	5×10^5	2
Ag_NP_3SO ₃ H	3×10^5	3
Ag_NP_6PO ₃ H ₂	3×10^5	3
Ag_NP_5COOH	3×10^5	3
Ag_NP_4COOH	0	4

and *tert*-butyltetra carboxypropoxy calix[4]arene **5**_{COOH} were synthesized as per Ohto [31] and purity confirmed by spectral means. The diphosphonate derivative, **6**_{PO₃H₂}, was synthesized as per the method of Markovsky and Kalchenko [32]; all spectral values are in accord with their values.

2.2. Nanoparticle Preparation and Characterization. The procedure of Xiong et al. [33] was slightly modified as follows. 10 mL of 1×10^{-2} M AgNO₃ solution was added to 80 mL of deionized water. To this solution, 10 mL of 1×10^{-2} M calix[n]arene aqueous solution was added as stabilizer with stirring for 30 min. And then, 44 mg of NaBH₄ was added to the solution. Then the calix[n]arene-capped silver colloidal

TABLE 3: The slopes of the variation of the maximum wavelength of Ag_NP_Calix[n]arene against the concentration of gentamycin sulfate.

Compounds	Slope (nm/M)	Rank
After 1 hour mixing with gentamycin sulfate		
Ag_NP_2SO ₃ H	9×10^6	1
Ag_NP_5COOH	9×10^6	1
Ag_NP_3SO ₃ H	2×10^6	2
Ag_NP_6PO ₃ H ₂	1.3×10^6	3
Ag_NP_1SO ₃ H	9×10^5	4
Ag_NP_4COOH	NA*	
After 24 hours mixing with gentamycin sulfate		
Ag_NP_2SO ₃ H	1.4×10^7	1
Ag_NP_6PO ₃ H ₂	1.3×10^7	2
Ag_NP_3SO ₃ H	3×10^6	3
Ag_NP_5COOH	1.2×10^6	4
Ag_NP_1SO ₃ H	1×10^6	5
Ag_NP_4COOH	NA*	

NA*: not applicable.

suspensions were characterized by UV-visible absorption assays. We monitored the change in absorbance between 340 nm and 650 nm, using a 96-titer well-visible spectrometer, (BioTek Power Wave 340).

2.3. Complexation Titration. All APIs were purchased from Sigma Aldrich; chlorhexidine (Merck index no. 55-56-1), chloramphenicol (Merck index no. 56-75-7) and gentamycin sulfate (Merck index no. 1403-66-3).

The complexation between API and calix[n]arene-capped silver colloidal suspensions was monitored with UV-visible absorption spectra after mixing for 1 hour and 24 hours.

FIGURE 2: The UV-visible spectra of Ag_NP_Calix[n]arene mixed for 1 hour with (a) 1×10^{-4} M chlorhexidine (b) 1×10^{-4} M chloramphenicol (c) 1×10^{-4} M gentamycin sulfate.

The calix[n]arene-capped silver colloidal suspensions are at a final concentration of 1×10^{-4} M. The API will be mixed at different final concentrations: 1×10^{-3} M, 1×10^{-4} M, 1×10^{-5} M, 1×10^{-6} M, and 1×10^{-7} M.

3. Results and Discussion

The chemical structures of the calix[n]arene derivatives are given in Scheme 1. The chemical structures of the APIs are given in Scheme 2 below.

The stability over the time of the different calix[n]arene-capped silver nanoparticles (Ag_NP_Calix[n]arene) solutions as characterized using UV-visible spectroscopy 1 hour and 24 hours after their preparation (Figure 1). Even if the intensity of absorbance decreases for some silver nanoparticle

solutions, in particular with Ag_NP_3SO₃H, Table 1 shows that the maximum wavelength of all Ag_NP_Calix[n]arene remains stable. This shows that silver nanoparticles are still present after 24 hours of their preparation. For all but Ag_NP_3SO₃H, the plasmon resonance absorption peak is sharp and centered at 390 nm; however in the case of Ag_NP_3SO₃H, the plasmonic peak is quite broad and centred at 420 nm suggesting a different assembly mode and also some aggregation.

Titration experiments were carried out between the six different types of calix[4]arene-capped nanoparticles and three APIs, chlorhexidine, chloramphenicol, and gentamycin.

Chlorhexidine is a clinically important antiseptic, disinfectant, and preservative. It is a potent membrane-active

FIGURE 3: The UV visible spectra of Ag_NP_Calix[n]arene mixed 24 hours with (a) 1×10^{-4} M chlorhexidine, (b) 1×10^{-4} M chloramphenicol, and (c) 1×10^{-4} M gentamycin sulfate.

agent against bacteria and inhibits outgrowth, but not germination, of bacterial spores. Chloramphenicol is considered a prototypical broad-spectrum antibiotic, alongside the tetracyclines. The most serious adverse effect associated with chloramphenicol treatment is bone marrow toxicity, which is rare (0.1% of the cases), unpredictable, unrelated to dose and in general fatal. As a consequence, it is no longer a first-line agent for any infection in developed nations, although it is sometimes used topically for eye infections. Nevertheless, the global problem of advancing bacterial resistance to newer drugs has led to renewed interest in its use.

Gentamycin sulfate is an aminoglycoside antibiotic, used to treat many types of bacterial infections, particularly those caused by Gram-negative organisms. Its bactericidal effect involves binding the 30S subunit of the bacterial ribosome, interrupting protein synthesis. Like all aminoglycosides, when gentamicin is given orally, it is not systemically active. This is because it is not absorbed to any appreciable extent

from the small intestine. It is administered intravenously, intramuscularly, or topically to treat infections.

The choice of the APIs was based on the facts that chlorhexidine is known to complex with all three types of calix[4]arene derivatives; the conformational flexibility of the molecule led to the presence of three distinct isomers in the solid state [34]. In the case of gentamycin it has already been shown that aminoglycosides complex with $1_{\text{SO}_3\text{H}}$ [10]; however for streptomycin the complexation was associated over several weeks with glycosidic bond cutting [35]. With regard to chloramphenicol, preliminary studies had shown a total lack of interaction and the molecule was chosen as a negative control.

Representative visible spectratitration curves at 1H and 24H are shown in Figures 2 and 3; for both gentamycin and chlorhexidine there are strong shifts in the plasmon resonance and intensity variations. For gentamycin in particular the changes in wavelength clearly arise from complexation on the nanoparticles by the API [28].

FIGURE 4: The delta values of the maximum wavelength of Ag_NP_Calix[n]arene as a matter of concentration of API mixed during 1 hour with (a) chlorhexidine and (b) gentamycine sulfate.

FIGURE 5: The delta of the maximum wavelength of Ag_NP_Calix[n]arene as a matter of concentration of API mixed during 24 hours with (a) chlorhexidine (b) gentamycine sulfate.

Given the large shifts in wavelength observed for the absorption associated with the plasmon resonance, the change in wavelength was plotted as a function of API concentration; see Figures 4 and 5.

For chloramphenicol, no change in the wavelength is observed; this is expected in view of the lack of complexation. For chlorhexidine and gentamycine, the curves show a typical concentration-dependent absorption associated with saturation. These reflect the different binding affinities of the various calix[4]arene derivatives with regard to the two APIs.

Given the incertitude in the exact concentration of complexation sites on the surfaces of the nanoparticles we consider it unwise to calculate association constants.

However comparative values can be extracted from the initial slopes of the curves and these are summarized in Tables 2 and 3.

There are clear variations in the affinity, while in general the hybrid silver nanoparticles capped with Ag_NP_2SO₃H show the highest affinity with chlorhexidine; after 1H the system is fourth in respect to affinity. Of the systems studied only Ag_NP_4COOH shows little or no affinity for these two APIs.

Thus we have observed selectivity in the interactions between the calix[4]arene capped nanoparticles and the APIs studied. This variation is dependent on the nature of the calix[n]arene, the API, and the kinetics of the interaction.

4. Conclusion

A series of novel calix[n]arene-capped silver nanoparticles have been prepared and their stability demonstrated. We have been able to couple calix[4]arenes having sulfonate, carboxylate, and phosphonate functions onto the silver nanoparticles. Tests with the APIs, chlorohexidine, chloramphenicol, and gentamycin sulfate, show strong affinity towards chlorohexidine and gentamycin, whereas for chloramphenicol little or no affinity exists. The affinities for the APIs depend on the nature of the calix[4]arene and on the nature of the API, and the kinetics of interaction vary as a function of both of the above.

Acknowledgments

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