Original Article

Characterization and haemocompatibility of *Aurum metallicum* for its potential therapeutic application

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Abstract

Background: The objective of the study was to characterize homoeopathic nanomedicine *Aurum metallicum* and evaluate its biocompatibility, to explore its possible application as injectables. Metal-based homoeopathic medicine, *Aurum metallicum,* was chosen as a model drug and the haemocompatibility of the drug at three different potencies 6C, 30C, and 200C were studied to find the justification of the drug as an injectable candidate for clinical application. **Methods:** The model drug *Aurum metallicum* at the three potencies was characterized by dynamic light scattering (DLS), zeta potential, field emission scanning electron microscopy (FESEM), and energy dispersive X-ray analysis. Hemocompatibility of the homoeopathic medicine was performed by haemolysis assay. Red blood cell obtained from fresh human blood by centrifugation was incubated with *Aurum metallicum*. Haemoglobin release was measured using UV-vis spectrophotometer at 540 nm. **Results:** The DLS and FESEM studies show a decrease of particle size with increasing potency. The zeta potential values show a fairly constant value measured at an interval of 10 days. The haemolysis percentage for 6C, 30C, and 200C was 9.73%, 8.16%, and 0.73%, respectively. **Conclusion:** The nanomedicine *Aurum metallicum* was nontoxic at all doses of 6C, 30C, and 200C. The haemolytic percentage also shows that 200C is nonhemolytic, showing haemolysis <2% as per the American Society for Testing and Materials guidelines. The undertaking of larger controlled and in-depth qualitative studies is warranted.

Keywords: Haemocompatibility, Homoeopathy, nanomedicine

Introduction

Inordinate use of antibacterial agents such as chemically modified natural compounds (penicillins and cephalosporins), pure natural products (aminoglycosides), and purely synthetic antibiotics (sulfonamides) lead to the development of drug-resistant microbes. These resistant pathogens cause the emergence of diseases, which are difficult to diagnose and control.[1] The causes of antibiotic resistance mainly include overuse, inappropriate prescribing, extensive agricultural use, availability of few new antibiotics, and regulatory barriers.[2] The Centers for Disease Control and Prevention has classified a number of bacteria as having urgent, serious, and concerning threats. Many of which are already responsible for placing a substantial clinical and financial burden on the US health care system, patients, and their families.[3-6] In 2010, India was the world's largest consumer of antibiotics for human health. The combination of factors such as poor public health infrastructure, rising incomes, a high burden of disease, and cheap, unregulated sales of antibiotics is causing such effect.[7-9]

Use of Homoeopathy has been looked upon as the potential answer for alternative therapy to microbes that are resistant to antibiotics. Studies suggested that homoeopathic remedies contain nanoparticles[10] which are heterogeneously dispersed in colloidal solution and act by modulating biological functions of cytokines, heat shock proteins and immune, endocrine, metabolic, autonomic, central nervous system functions, etc.[11-16]

In general, there are several methods of introducing homoeopathic drugs into the body for remedial purposes.

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According to Bergmann *et al*., 1917, it is advantageous for the homoeopathic remedy if it does not have to pass through the gastrointestinal tract where it is severely altered by the gastric and intestinal juices. The potentized homoeopathic remedies work best when applied unchanged to the mucosa or - even better -injected under the skin or into the bloodstream.[17] Subcutaneous and intravenous administrations were the first parenteral forms of administrations used in Homoeopathy. Others forms of administrations are intramuscular, intracutaneous, intra-articular, and periarticular. Baars *et al*., 2003, discussed about the risks of the subcutaneous form of administration of homoeopathic medicine. Bell *et al*., 2011, discussed about the short-term effects of repeated olfactory administration of homoeopathic *Sulphur* or *Pulsatilla*. Cazin *et al*., 1987, studied the intraperitoneal injection of decimal and centesimal dilutions of *Arsenicum album* on the retention and mobilization of arsenic in rat and concluded that arsenic excretion is primarily fecal for the period of 12–20 h and among the dilutions studied most active dilution was 14dH and 7cH.[18-20] Hahnemann instructed that the tongue, mouth, and stomach are the most effective routes of administration for this purpose. Recently, medicinal solutions, manufactured in accordance with the German Homoeopathic Pharmacopoeia, are therapeutically applied as injections for better results for a wide range of conditions, especially in the treatment of acute and chronic diseases.[21,22]

It is necessary that the selected homoeopathic drug should be compatible with blood cells and must not cause any damage or haemolysis of blood cells. We have reported here our study on the effect of a homoeopathic medicine *Aurum metallicum* at various potencies (6C, 30C, and 200C) on haemolysis. Our result indicates that the medicine is haemocompatible and the magnitude of this effect depends on the potency of the medicine.

Dynamic light scattering (DLS) and field emission scanning electron microscopy (FESEM) studies for characterization of the drug at three potencies including the potentized and unpotentized alcohol *Aurum metallicum* were performed to establish its role as potential nanomedicine.

Materials and Methods

Materials

The drug *Aurum metallicum* was obtained from Hahnemann Publishing Company, India and was used without further processing. Sodium chloride and Na_2-EDTA were purchased from E. Merck (India).

Methods

Field emission scanning electron microscopy and energy dispersive X-ray analysis

Morphological characteristics of *Aurum metallicum* at different potencies (6C, 30C, and 200C) were observed by FESEM (Model FEI Quanta 250, USA). A small amount of sample was cast on a clean glass coverslip and was directly placed on carbon-coated grid, which was then sputter coated with gold and then observed by FESEM. The particle sizes were calculated using ImageJ software, NIH, USA.

For energy dispersive X-ray analysis (EDAX), the *Aurum metallicum* samples (6C, 30C, and 200C) including their succussed and unsuccussed control were placed on carbon grid and placed directly without gold coating.

Dynamic light scattering and zeta potential

DLS measurements of *Aurum metallicum* samples at different potencies (6C, 30C, and 200C) were performed using a Nano-ZS 90-Malvern instrument (Model DLS-nano ZS, Zetasizer, Nano series, USA) employing a 4 mW helium–neon laser (l¼ 632.8 nm) equipped with a thermostatic sample chamber. The zeta potential measurement of *Aurum metallicum* (6C, 30C, 200C) and their succussed and unsuccussed control was repeated thrice in an interval of 10 days, and the data were presented by average value ± standard error of the mean (SEM).

Haemolysis assay

Five milliliters of venous blood was drawn from three healthy human volunteers, under the proper medical supervision and collected in a Na-EDTA vial (1.8 mg/ml). Blood was mixed by gentle inversion of the tube and centrifuged at 1200 ×*g* for 10 min. The plasma supernatant was discarded, and the red blood cells (RBCs) were washed three times by suspending them in normal saline (0.9%). The final suspension consisted of 5% by volume RBC in saline.[23]

Fifty microliters of each of the *Aurum metallicum* potencies (6C, 30C, and 200C) was dried in 2 ml microcentrifuge tube in a vacuum desiccator. Fifty microliters of normal saline (0.9%) was added to all the tubes. Finally, 200 µl of RBC solution was added. Deionized water and 0.9% saline in the same volume served as negative and positive control, respectively. Samples were incubated at 37°C for 1 h. After incubation, the tubes were centrifuged at 1200 g for 10 min, and absorbance was taken at 540 nm. Haemolysis percentage was calculated from the formulae:

$$\%\ \text{Haemolysis} = \frac{\text{Absorbance of the test sample} - \text{Absorbance of negative control}}{\text{Absorbance of the positive control} - \text{Absorbance of negative}} \times 100$$

A substance is considered to be haemocompatible if the haemolysis percentage is < 10%.[24] The results are represented as the mean ± standard deviation of the three independent experiments.

Results and Discussion

Electron micrographs

The FESEM images of *Aurum metallicum* are shown in Figure 1a-c. As seen in the figure, particles in nano dimensions are embedded in lactose matrix, which is used for the preparation of homoeopathic drugs. Further, with a gradual increase of dilution from 6C to 30C to highly diluted 200C, the size of the nanoparticles decrease.

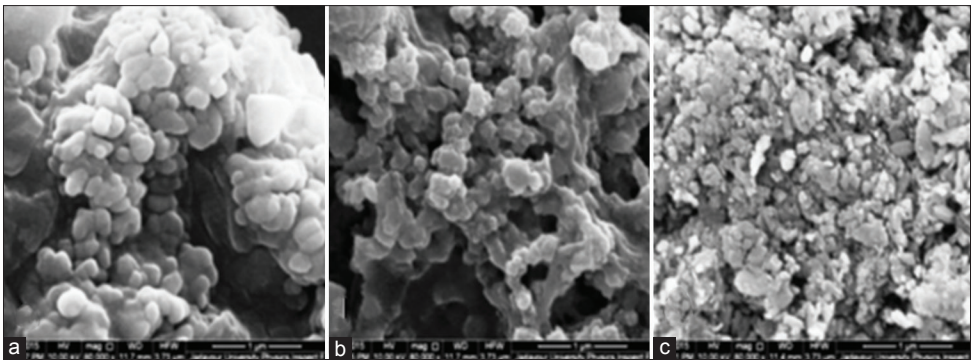

Figure 1: Field emission scanning electron microscopy images of *Aurum metallicum* prepared by drop casting followed by drying (a) 6C (b) 30C (c) 200C. Scale bar represents 1 μm

Particles are also seen to be agglomerated possibly due to the drying process during sample preparation. The average particle sizes of the homoeopathic medicine are represented in Table 1. The approximate size calculated from ten particles for 6C, 30C, and 200C are 268.15, 171, and 100 nm, respectively. The presence of nanoparticles of the gold, copper, tin, zinc, silver, and platinum present in *Aurum metallicum*, *Cuprum metallicum*, *Stannum metallicum*, *Zincum metallicum*, *Argentum metallicum,* and *Platinum metallicum,* respectively, at 6C, 30C, and 200C potency was demonstrated by Chikramane *et al*., 2010.[25] Their group reported the presence of nanoparticle of the starting material in the extreme dilutions of 6C, 30C, and 200C.[26] The work of Chikramane *et al*., 2012, does not show any dependence of size of nanoparticle on potency.[27] However, our results (both FESEM and DLS) categorically show this dependence. Using four other homoeopathic medicines, we have been able to derive an empirical relation between the size and potency of drug particles.[28]

The preparation of homoeopathic nanomedicine mainly consists of two steps. The trituration with lactose followed by succession step, in which the triturated preparations are potentized with alcohol in glass container whereby the particles develop a coat of silica. These particles were seen embedded in a meso-microporous silicate layer through interfacial encapsulation. Thus, metal and inorganic salt-based homoeopathic medicines retain the starting material as nanoparticles encapsulated within a silicate coating.[29-32] Our study also shows the presence of silica as well as gold shown by EDAX analysis [Table 2].

Dynamic light scattering studies

DLS is one of the most established particle-sizing techniques that use the light scattered by particles to determine their size, in terms of the hydrodynamic diameter.[33] Drawbacks of DLS include its intrinsic propensity to detect larger particles. It is considered less suitable for characterizing heterogeneous systems.[34] The particle size distribution of the *Aurum metallicum* shows a decrease in particle size with increase in potency [Figure 2a-c]. The particle size for 6C, 30C, and 200C is, respectively, 295.3 nm, 88.98 nm, and 10.3 nm. The DLS data validate the trend observed in the FESEM images [Table 1]. It is also noted that the size observed in SEM is slightly more than that as seen in the DLS size distribution.

Table 1: Comparative of dynamic light scattering and field emission scanning electron microscopy of *Aurum metallicum* for different potencies

Potency	DLS	FESEM
6C	295.3-396.1	268.1514±36.433
30C	88.98-162.4	171.6795±25.548
200C	10.03-88.82	100.0009±5.190

DLS: Dynamic light scattering; FESEM: Field emission scanning electron microscopy

Table 2: Elemental analysis by energy dispersive X-ray analysis represented by mass percentage of *Aurum metallicum* (6C, 30C, 200C) and potentized and unpotentized alchohol

Element	Mass percentage				
	Alcohol		*Aurum metallicum*		
	Unpotentized	Potentized	6C	30C	200C
Oxygen	100	53.26	45.74	15.34	38.71
Gold	-	-	14.12	71.21	33.97
Silicon	-	46.74	40.14	13.45	27.32

This may perhaps be due to the fact that preparation for SEM causes agglomeration with respect to the dispersed form, causing the increase in the particle size.

The zeta potential values are represented in Table 3. As seen in the table, the values were fairly constant when recorded at an interval of 10 days representing the stable nature of the particle. Moreover, it is to be noted that the value of zeta potential increases after succession and further increases after the addition of metal. The data support that homoeopathic medicines *Aurum metallicum* were more stable than their succussed and unsuccussed controls.

Haemocompatibility

The haemolysis percentage of 6C, 30C, and 200C was found to be 9.73%, 8.16%, 0.73%, respectively [Figure 3]. There

Table 3: Zeta potential of *Aurum metallicum* (6C, 30C, 200C), unpotentized and potentized alcohol

Sample	6C	30C	200C	Unpotentized	Potentized
Zeta potential (Mv)	−2.30±0.25	−4.08±0.49	−2.88±0.55	−0.81±0.54	−1.28±0.33

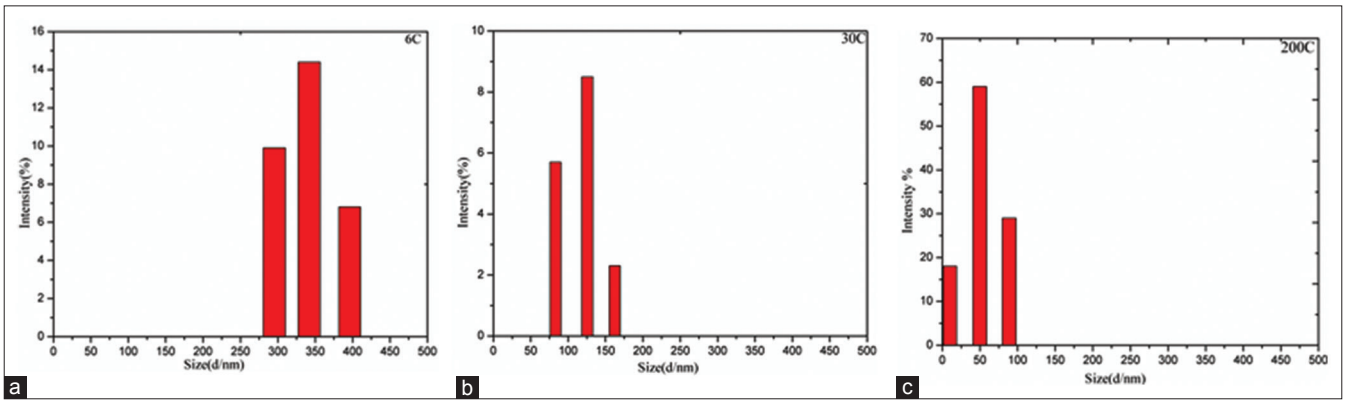

Figure 2: Hydrodynamic diameter distribution plots as determined by DLS measurements of *Aurum metallicum* (a) 6C (b) 30C (c) 200C

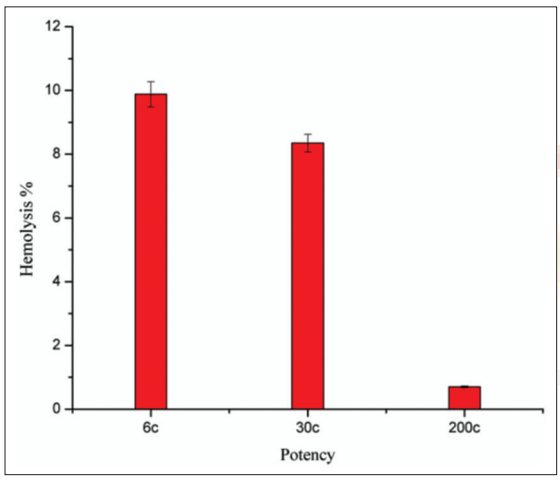

Figure 3: Haemolysis percentage and potency of *Aurum metallicum* (values ± standard error of the mean)

are several views available in the literature regarding the limit percentage of haemolysis and toxicity. Some authors opine limit of nonhemolytic percentage as 10%.[23,24] Accordingly, our result signifies that *Aurum metallicum* is nontoxic toward RBC at 6C, 30C, and 200C potencies. While according to the American Society for Testing and Materials guidelines, a material is classified as nonhemolytic, slightly hemolytic if the haemolytic index is <2%, 2%–5%, and haemolytic if >5%, respectively. As per their guidelines, homoeopathic nanomedicine *Aurum metallicum* is nonhemolytic at the 200C potency (0.73%).

Conclusion

The size of the drug particles of *Aurum metallicum* used in this study at three potencies are found to be in the nanometer range where the particle size decreases with increase in potency. All the samples at different potencies are found to be nontoxic, causing minimum harm to the RBCs. However, out of these three potencies, hemolysis is maximum for the drug at 6C potency. This result can be justified from our previous study of measurement of fluidity of artificial lipid membrane of dipalmitoylphosphatidylcholine with different potencies of another metal-derived homoeopathic medicine *Cuprum metallicum* where it has been observed that the drug at 6C potency fluidizes the membrane most followed by 30C and 200C.[13] The decrease in membrane anisotropy with increasing potency was further explained by the fact that with an increase in potency, not only the particle size reduces but also the number of drug particle decreases. Therefore, the possibility of interaction of the drugs of higher potency (200C) with the RBC membrane reduces, causing less damage to the membrane than the drug at higher potency (6C and 30C). Hence, from the study, we may conclude that homoeopathy drug *Aurum metallicum* has a potential scope in future medicine in the injectable form. More in-depth studies are required to understand the interaction of homoeopathic medicine with RBC and other blood cells. Further, *in vivo* studies will throw more light toward the development of nanomedicine as injectables.

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Conflict of interest

There are neither any financial nor any personal conflict of interest with respect to the work carried out for this article.

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Charakterisierung und Hämokompatibilität von Aurum metallicum für seine potenzielle therapeutische Anwendung

Auszug

Hintergrund: Das Ziel der Studie war es, die homöopathische Arznei Aurum metallicum zu beschreiben und deren Biokompatibilität zu untersuchen, um ihre mögliche Anwendung als Injektionsmittel zu untersuchen. Die auf einem Metall basierende homöopathische Medizin, Aurum metallicum, wurde als Versuchsarznei und deren Hämokompatibilität mittels dreier verschiedener Potenzen (C6, C30 und C200) untersucht, um eine Anwendung des Arzneimittels als Iniectabila für die klinische Anwendung zu finden.

Methoden: Die Versuchsarznei Aurum metallicum in den drei Potenzen wurde mittels dynamischer Lichtstreuung (DLS), Zeta-Potential, FESEM und EDAX gekennzeichnet. Die Hämokompatibilität der Arznei wurde mit dem Hämolysetest durchgeführt. RBC, aus frischem menschlichem Blut durch Zentrifugation gewonnen, wurde mit Aurum metallicum inkubiert. Die Hämoglobinfreisetzung wurde unter Verwendung eines UV-Vis-Spektrophotometers bei 540 nm gemessen.

Ergebnisse: Die DLS- und FESEM-Studien zeigen eine Abnahme der Partikelgröße mit steigender Potenz. Die Zeta-Potentialwerte zeigen einen relativ konstanten Wert, der in einem Intervall von 10 Tagen gemessen wird. Die Hämolyse in% für C6, C30 und C200 betrug 9,73, 8,16 bzw. 0,73%.

Fazit: Aurum metallicum war in allen Dosen von C6, C30 und C200 nicht toxisch. Der hämolytische Prozentsatz der Hämolyse zeigt auch, dass C200 nicht hämolytisch ist und eine Hämolyse von weniger als 2% nach den ASTM-Richtlinien aufweist. Die Durchführung größerer kontrollierter und eingehender qualitativer Studien ist gerechtfertigt.

Caracterización y hemocompatibilidad de Aurum Metallicum para su aplicación terapéutica potencial

RESUMEN

Fundamento: El objetivo de este estudio fue caracterizar el nanomedicamento homeopático Aurum metallicum y evaluar su biocompatibilidad para explorar su posible aplicación como inyectable. Se eligió Aurum metallicum, un ´remedio homeopático metálico, como modelo medicamentoso y se estudió su hemocompatibilidad a tres diferentes potencias 6C, 30C y 200C para establecer la justificación del medicamento como inyectable en la aplicación clínica.

Métodos: El modelo medicamentoso, Aurum metallicum, a tres potencias se caracterizó por dispersión de luz dinámica (DLS), potencial Zeta, FESEM y EDAX. La hemocompatibilidad del medicamento homeopático se realizó con un ensayo de hemolisis. Los glóbulos rojos de sangre fresca humana, obtenidos por centrifugación, se incubaron con Aurum metallicum. La liberación de hemoglobina se midió utilizando un espectrofotómetro UV-vis a 540nm.

Resultados: Los estudios de DLS y FESEM mostraron un descenso del tamaño de partículas conforme aumentaba la potencia. El potencial zeta mostró un valor bastante constante medido a un intervalo de 10 días. La hemolisis porcentual de 6C, 30C y200C se situó en un 9,73, un 8,16 y un 0,73%, respectivamente.

Conclusiones: El nanomedicamento Aurum metallicum no fue tóxico a ninguna de las dosis de 6C, 30C y 200C. El porcentaje hemolítico muestra que la 200C es no hemolítico, al presentar una hemolisis inferior al 2% conforme a las directrices ASRM. Queda justificada la realización de estudios controlados y altamente cualitativos más amplios.

संभावित चिकित्सीय उपयोग के लिए ऑरम मेटालिकम की विशेषताएँ और रूधिरानुकूलता।

सार

उद्देश्य: होम्योपैथिक नैनो औषधि ऑरम मेटालिकम की विशेषताओं और इसकी जैव अनुकूलता का मूल्यांकन तथा इंजेक्शन के रूप में इसके संभावित उपयोग का पता लगाना था। धातु आधारित होम्योपैथिक औषधि, ऑरम मेटालिकम का चयन एक मॉडल औषधि के रूप में किया गया। नैदानिक उपयोग में इंजेक्शन के रूप में इसकी रूधिरानुकूलता के औचित्य का अध्ययन तीन अलग–अलग मात्राओं 6सी, 30सी और 200सी में किया गया।

विधि: तीन पोटेंसीज़ में मॉडल औषधि ऑरम मेटालिकम का डाइनामिक लाईट स्केटरिंग (डीएलएस), ज़ीटा पोटेंशियल, एफइएसइएम और इडीएएक्स द्वारा गुण–चित्रण किया गया। होम्योपैथिक औषधि की रूधिरानुकूलता, रक्तापघटन परख द्वारा प्रदर्शित की गई। अपकेंद्रित द्वारा मानव रक्त में प्राप्त लाल रक्त कोशिकाओं के साथ ऑरम मेटालिकम का ऊष्मायन किया गया। उत्सर्जित हीमोग्लोबिन को 540 एनएम पर यू.वी. विज़ स्पेक्ट्रोफोटोमीटर का उपयोग कर मापा गया।

परिणाम: डीएलएस ओर एफइएसइएम अध्ययन पोटेंसिज़ में वृद्धि के साथ कण आकार में कमी दर्शाते हैं। ज़ीटा संभावित मूल्यों में 10 दिनों के अंतराल में मापन पर लगातार उचित मूल्य दर्शाए गए। 6सी, 30सी और 200सी के लिए रक्तापघटन प्रतिशत क्रमशः 9.73, 8.16 और 0.73 प्रतिशत थे।

निष्कर्ष: नैनो औषधि ऑरम मेटालिकम सभी 6सी, 30सी ओर 200सी खुराकों में गैर विषैला था। रक्तलायी प्रतिशत द्वारा यह भी पता चलता है कि 200सी, गैर रक्तलायी है, जो एएसटीएम के दिशा निर्देशों के अनुसार 2 प्रतिशत से कम प्रदर्शित था। अधिक नियंत्रित और गहन गुणात्मक अध्ययन के उपक्रम आवश्यक है।