Nanogold-pharmaceutics

(i) The use of colloidal gold to treat experimentally-induced arthritis in rat models; (ii) Characterization of the gold in *Swarna bhasma*, a microparticulate used in traditional Indian medicine

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Abstract

Nanosized gold particles (27 +/- 3 nm) have been proven to be effective in ameliorating the symptoms of mycobacterial-, collagen- and pristane-induced arthritis in rat models. This contrasts with the drug sodium aurothiomalate that was only effective against mycobacterial-induced arthritis but not to the same extent as Au^0. Gold in the traditional Indian Ayurvedic medicine, *Swarna bhasma* (gold ash), has been characterized as globular particles of gold with an average size of 56-57 nm.

Keywords

Gold, nanoparticle, experimental arthritis, ayurvedic medicine, aurothiomalate

Introduction

The use of gold and gold compounds, as well as their potential therapeutic applications, in ancient and contemporary medicine has been reviewed periodically over the years [1-13]. At present, the most active area in developing gold-based pharmaceuticals is their investigation as potential anti-tumour agents [9]. Thus, both gold(I) [14, 15] and gold(III) [16-18] compounds are being actively studied in this regard. Gold compounds have also been investigated for anti-HIV activity [19-21], as anti-malarial agents [22] and even for the treatment of bronchial asthma in Japan [23-25]. However, the principal contemporary use of gold in medicine, *chrysotherapy*, still remains for treating arthritis: the first focus of this report.

Gold drugs comprise a class of distinctive anti-arthritic agents (Disease-Modifying Anti-rheumatic Drugs, the DMARDS) used when non-steroidal anti-inflammatory drugs (NSAIDS) such as Advil® (ibuprofen), Naprosyn® (naproxen), and Voltaren® (diclofenac sodium) are insufficient to treat severe cases of rheumatoid or psoriatic arthritis. They are often used as a last resort in treating patients. The two most commonly used gold drugs are the polymeric charged sodium aurothiomalate (**I**, Myocrosin®) and, the more recently available monomeric neutral auranofin Ridaura® (**II**, also known as Auranofin®), Scheme I. Initially, (**I**) was introduced for the treatment of infectious diseases such as tuberculosis and then subsequently used for the amelioration of rheumatoid arthritis (RA) that afflicts between 1 – 2 % of the population in industrialised nations. Both a) the side-effects of *chrysotherapy, e.g.,* dermatitis, nausea, bone marrow damage, *etc.*, inconvenience of administration, *e.g.,* intramuscular injection for (**I**) and b) lack of drug company support have led to ever diminishing of *gold* use today – this, despite the fact there are very few other therapies which can emulate gold for inducing arthritis remission. Pharmaceutical companies may not necessarily be prejudiced against "gold" but, certainly, they do not promote gold therapies - in fact, many former suppliers have ceased to manufacture gold drugs. Reasons are not hard to find such as i) no blockbuster market to justify high promotional costs and ii) no strong patentable base to minimize competition. The new 'alternatives' to gold, particularly the biological DMARDs, do a lot of things that the gold drugs don't: promote infectious diseases (TB, HIV), allow lymphoma development, and exclude the less wealthy population from benefits of therapy, to name the more obvious [26]. Many reports are now becoming available that indicate these pharmaceuticals are no more than palliative *e.g.* minimising responses to tumour necrosis factor (TNF) but not undercutting what causes too much TNF to be produced in the first place. This contrasts with gold therapy where reported remissions of rheumatoid arthritis seem to be of the order of 30 per cent, perhaps even greater in some very traditional European clinics [27].

While the precise mechanism of action of gold drugs is not known, it is quite clear that the *gold* content is pivotal for therapeutic benefit, indicating that (**I**) and (**II**) are *pro-drugs;*

Table 1

*Comparing anti-arthritic effects of parenteral sodium aurothiomalate (**I**) and Tyndall's purple (Au^0) in three types of experimental polyarthritis in two strains of rats*

Arthritis induced with	Rat strain	Mean arthritis scores* with			Measured on day**
		No treatment	ATM	Au°	
Mycobacterium	Wistar	2.3+	0.5+	0.3+	15
Tuberculosis	Dark Agouti	1.8+	0.2+	0.8+	12
Collagen Type-II	Wistar	1.7+	1.8+	0.2+	15
	Dark Agouti	1.5+	1.3+	0.3+	32
Pristane	Wistar	2+	1.8+	0.3+	18
	Dark Agouti	1.8+	nd	0.5+	15

*Scores compiled for each animal (on a scale 0-4+) after measuring rear paw and tail swelling, and assessing fore paw inflammation and general well-being (mobility, grooming, behaviour, etc.).

[Two independent observers made these assessments.]

**Day of assessment

nd = not determined

i.e. the administered species being transformed/metabolized to release *gold in vivo*.

Some of the side-effects of *chrysotherapy* may relate to the generation of gold(III). Monovalent gold(I), as in (**I**) and (**II**), dismutates to gold(III) ions and metallic gold (Au^0). Gold(III), like other heavy metals, denatures proteins and nucleic acids, and may be responsible for much of the toxicity of administered gold(I) drugs [28, 29]. On the other hand, metallic gold has been generally regarded as biologically inert, though it may constitute the electron-dense material accumulating in lysosomes ('aurosomes') after the injection of gold(I) preparations.

In order to minimize deleterious side-effect while retaining efficacy of gold therapy, we have explored the pharmacological potential of nano-sized gold particles in rats with experimentally-induced arthritis. Our preliminary findings of this on-going investigation are reported herein. Nanoparticles of gold are attracting increasing attention in nanotechnology but applications in medicine are still relatively unexplored [30, 31].

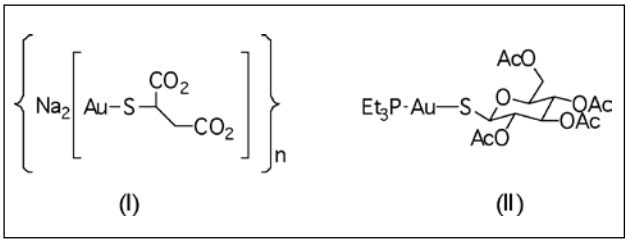
Gold preparations have been used for therapeutic benefit by Arabic, Chinese and Indian cultures as far back as 2500 B.C. [3, 8]. Within the context of traditional Indian Ayurvedic medicine, *Swarna bhasma* (gold ash) has been used as a therapeutic agent for several clinical disorders including bronchial asthma, rheumatoid arthritis, diabetes mellitus, nervous diseases, *etc.* [32-34]. *Swarna bhasma* is given orally to patients, usually mixed with honey or cow ghee (a preparation made by heating butter). Ayurvedic doctors insist that in this way gold particles get absorbed through sublingual route directly into blood stream like a homeopathic drug. However, there is no experimental proof available. The antioxidant/restorative effects of *Swarna bhasma* against global and focal models of Ischaemia (stroke) have been reported in modern times [33]. Very little is known about the chemical composition of *Swarna bhasma* [35], let alone the scientific basis of its application in various diseases. At least twenty different methods for preparing *Swarna bhasma* are described in various Ayurvedic texts based on incineration with mercury, mercury sulphide, sulphur, orpiment (As_2S_3), realgar (AsS), chalcopyrite, *etc.* The preparation based on incineration with mercury is considered to provide the best quality. Therefore, a study was initiated for standardizing a *Swarna bhasma* preparation (incineration with mercury), its physicochemical characterization and clinical evaluation. The characterizations of *Swarna bhasma* and of the gold particles therein are reported here.

Experimental

A standardised colloidal gold preparation (Tyndall's purple) was prepared by reduction of chloroauric acid with sodium citrate at 22°C [36]. The average particle size of the gold nanoparticles was 27 +/- 3 nm. Absorption spectrometry (525 nm) indicated no change in composition over six months when suspensions were stored at 4°C.

To evaluate gold drugs, three forms of experimental arthritis were established in rats as follows:

- Mycobacterial-induced arthritis [37] was initiated by tailbase injection of 0.6 mg heat-killed delipidated *Mycobacterium tuberculosis* administered in 0.1 ml squalane.
- Collagen-induced arthritis [38] was induced with tailbase injection of 200 µg bovine cartilage collagen emulsified



Scheme 1

with Freund's incomplete adjuvant and given in several divided doses.

- Pristane-induced arthritis [39] was initiated by tailbase injection 200 µl pristane ($C_{19}H_{40}$) in divided doses.

Gold preparations were administered subcutaneously to groups of six or seven rats every second day beginning on the day when these arthritigens were injected. Myocrisin (Rhone-Poulenc Rorer), dissolved in isotonic NaCl (150 mM) was given at a dose of 3 mg/kg gold. Colloidal Au^0, dispersed in isotonic sorbitol (0.3 M) was given at a dose of 3.3 µg/kg gold. Signs of arthritis were assessed from day-11 onwards by measurements of tail and paw swelling and assigning an overall arthritis score to each animal. Results are summarized in Table 1. Animals cannot be kept for more than 25 days under the protocols of the Animal Ethics Committee at GU which means animals cannot be kept indefinitely to study how long drug action is durable.

Preparation of *Swarna bhasma*

The procedure for purification was as per *Rasratnasamucchaya* [40] and for incineration, the procedure followed was from *Sarangadhara-Samhita* [41].

General purification

Pure gold (10 g, 24 k) was pressed into a foil of 40 – 60 µm thickness with the aid of a foil-making machine. Purification (*Sāmanya Shodhan*) was carried out by dipping the red-hot gold foils in sesame oil (*Sesamum indicum* Linn.), this process being repeated seven times. After each dipping of the gold foil, the oil was replaced by a fresh sample. The foils were then processed in the same manner sequentially using butter milk, cow's urine, Kanji (sour gruel processed from rice, *Oryza sativa*) and a decoction of Kulatha (*Dolichos biflorus* Linn.). The process was repeated seven times in each liquid. Finally the gold leaves were dried and weighed. No significant changes were observed in weight or physical appearance, except the metallic shine seemed brighter.

Special purification

Special purification was achieved by applying a paste of hematite and rock salt on gold foil and heating it in an earthen crucible for 1.30 h over strong heat (~1000°C). The material was allowed to cool.

Process of incineration

Purified gold foils were cleaned thoroughly and cut into very small pieces. The small pieces (approx. 10 g) were added to 20 g of pure mercury, placed in a stone mortar pestle and rubbed. The amalgam formed was placed into an earthen crucible along with sulphur in a 1:1 proportion. The crucible was covered by placing another inverted earthen crucible on top of it and sealed by three layers of cotton cloth and wet clay. The assembly was heated for 8 h inside cow dung cakes (four, 250 g each) in a pit. The maximum temperature during heating was ~900°C. After cooling, the incinerated matter was ground and the same procedure of heating with sulphur (1:1 proportion) was repeated. The procedure of grinding, heating with sulphur and cooling was repeated 42 times to obtain a homogenous brown red powder, i.e., *Swarna bhasma*.

Physicochemical characterization of Swarna bhasma

Atomic Absorption Spectroscopy: An Atomic Absorption Spectrometer (Perkin Elmer, USA) was used for quantitative analysis of gold in the *Swarna bhasma*. *Swarna bhasma* (10 mg) was digested in 2 ml of *aqua regia* and after complete digestion, the volume of the solution was made up to 25 ml with distilled water. Appropriate dilutions were made and the concentration of gold was determined by flame AAS. As the *Swarna bhasma* preparation involved addition of mercury, the sample was also analysed for mercury content by the cold vapour method of AAS.

FT Infrared Spectroscopy: Samples were investigated as KBr disks on a Perkin Elmer Spectrum One FTIR spectrophotometer.

Transmission Electron Microscopy: Transmission Electron

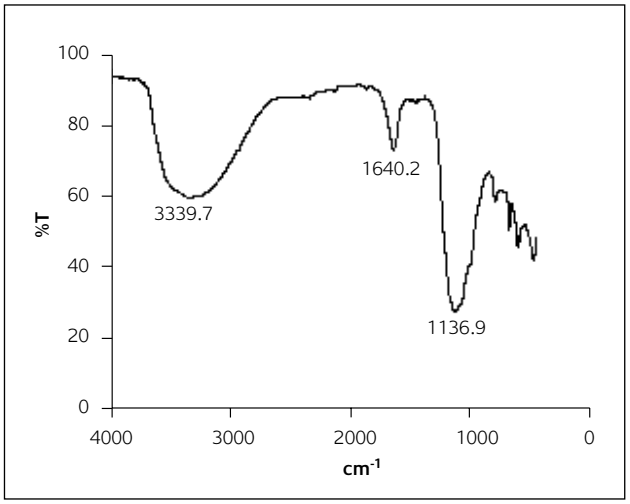


Figure 1
The infrared spectrum of Swarna bhasma *(KBr Disk)*

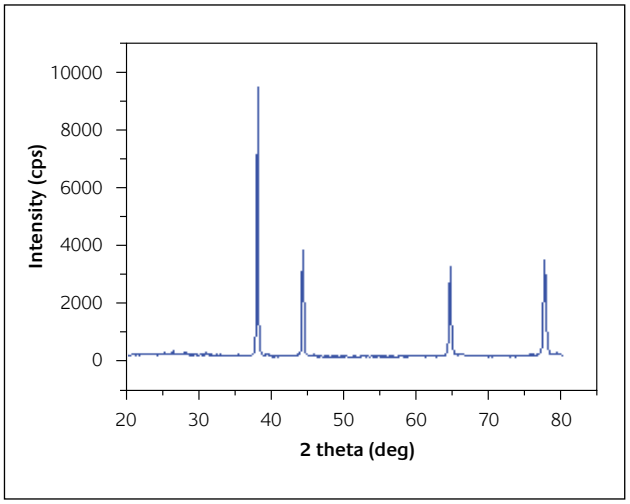


Figure 2
XRD pattern recorded for Swarna bhasma

Microscope (Zeiss 109, Germany) was used for particle size estimation of *Swarna bhasma*. The *Swarna bhasma* (50 mg) was suspended in distilled water (2 ml). The suspension was diluted 10 times and a drop of this solution was put over a formavar coated copper grid. The grid was dried at 70°C. The sample was analyzed under the TEM at magnification 140000X and accelerating voltage 60 kV.

Atomic Force microscopy: The *Swarna bhasma* (10 mg) was suspended in distilled water and after appropriate dilution a drop (~10 µl) of the solution was put over a cover slip and dried. AFM imaging was performed on a Nanonics MultiView 1000 AFM head with E scanner (Nanonics Imaging Ltd., Jerusalem, Israel). Scanning was performed in tapping mode. Images were obtained with 20 nm radius AFM tips obtained from Nanonics Imaging. The cantilever is oscillated at its free resonance frequency (typically 80 kHz). The exact position of the tip onto the sample was controlled using an inverted microscope (Olympus, Japan) mounted above the AFM. All measurements were performed at ambient temperature (20°C) in air. AFM images were captured, processed and analyzed with QUARTZ software, version 1.00 (Cavendish Instruments Ltd., UK).

X-Ray Diffraction Analysis: The XRD powder diffraction pattern of *Swarna bhasma* was recorded on X-ray diffractometer (Bruker AXS, D8 advance series, Germany) using CuKα radiation, λ = 1.5406 Å, filtered by nickel foil over the range 20.0 – 80.0°.

Results and discussion

Anti-arthritic activities of colloidal gold

The standardized preparation of Au^0 was considerably more potent, by approximately 10^3 times, than the reference drug employed in this study sodium aurothiomalate (**I**), when both were administered subcutaneously. The results obtained in this study are summarized in Table 1. At this very low level, Au^0 was ineffective when given orally; probably because it coagulates in the presence of gastric juice enteric sodium chloride. It was found to be important to administer Au^0 subcutaneously in a low-salt but isotonic solution to minimise particle aggregation.

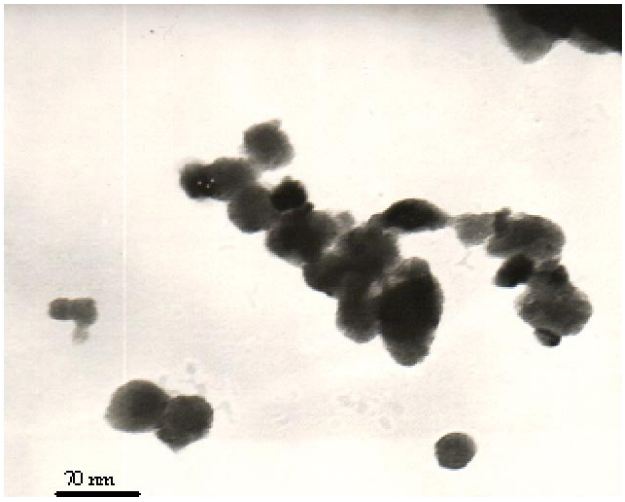


Figure 3
TEM image of Swarna bhasma *shown at a magnification of 1,40000X*

Colloidal Au^0 inhibited the development of all three forms of experimental arthritis, in contrast to sodium aurothiomalate (I) which was only effective against the mycobacterial (adjuvant)-induced arthritis. Even in this particular model arthritis, the efficacy of sodium aurothiomalate (I) may depend on the rat strain used [37] – indicating some genetic factors may control the expression of anti-arthritic activity. By contrast, the anti-arthritic activity of the colloidal Au^0 was far broader, controlling disease development regardless of which arthritigen was used to establish the polyarthritis. Finally, it is noted that the anti-arthritic effects of these gold preparations are long-lasting after ceasing treatment, a result in contrast to very many drugs currently used for treating arthritis whose effects are transient or relatively short-lived in these experimental arthritides.

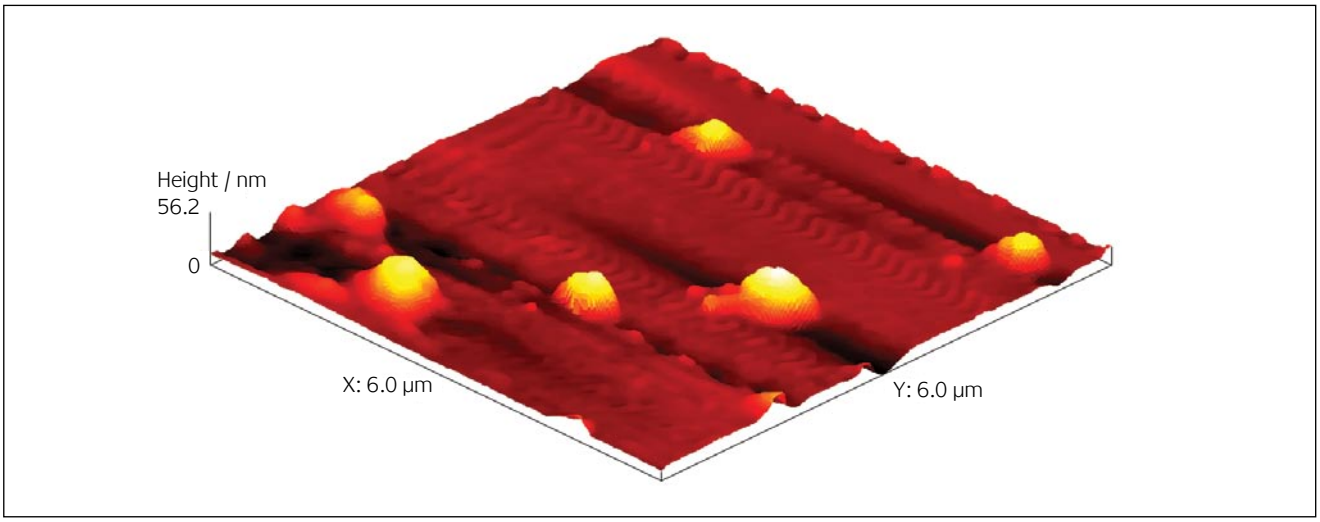


Figure 4
Atomic Force Microscopy image for Swarna bhasma

Characterization of *Swarna bhasma*

Swarna bhasma was prepared following literature procedures (see Experimental) was shown to contain 92% gold by Atomic Absorption Spectroscopy. However, mercury was not detected in the sample of *Swarna bhasma*. Often, the acceptability of Ayurvedic medicines in Western countries is limited due to the presence of toxic metals notably lead, arsenic and mercury. The absence of mercury in the present *Swarna Bhasma* preparation indicates proper incineration yielding a high-quality product with better potential acceptability.

The infra-red spectrum of *Swarna bhasma*, Figure 1, showed that it contained no organic compounds. The observed absorptions correspond to inorganic metal, hydrated metal salt or oxide. The absence of organic matter is further proof of proper incineration during the preparation of *Swarna bhasma* and the absence of any external organic contamination. This conclusion accords with an earlier infra-red study [35] and is further borne out by the XRD study.

The XRD pattern of *Swarna bhasma* is shown in Figure 2. The diffraction peaks at 2θ = 38.2°, 44.4°, 64.6° and 77.6° are identical with those reported for the standard gold metal (Au^0) (JCPDS, USA). No other diffraction peaks were observed confirming that the drug *Swarna bhasma* is composed of mainly gold nanoparticles. The high intensity of XRD lines in the XRD pattern suggests that the drug is present in crystalline form. The size of gold crystallites in *Swarna bhasma* was calculated from the XRD pattern using the Scherrer formula and determined to lie in the range 23 - 37 nm. The Scherrer equation is not always a reliable measure of particle size; in fact sharp peaks indicate slightly higher sizes. Hence, other forms of characterisation were explored.

An image from the Transmission Electron Microscopic study is shown in Figure 3. This indicated the average particle size of *Swarna bhasma* to be 57 nm with globular morphology. The globular nature of *Swarna bhasma* was confirmed by Atomic Force Microscopy, Figure 4, with an average particle size of 56 nm.

The results of microscopy and XRD confirmed our hypothesis that *Swarna bhasma* particles might be in the nanometer size domain.

Danscher demonstrated the *in vivo* liberation of gold ions from gold implants [42]. In another recent study, Hillyer and Albrecht [29] demonstrated that uptake of gold nanoparticles occurred in the small intestine by persorption through single, degrading enterocytes in the process of being extruded from a villus and gold nanoparticles typically less than 58 nm in size reach various organs through blood. Since gold in the *Swarna bhasma* prepared in the present study was predominantly zerovalent and approximately 56-57 nm in size, it is possible that it would reach the affected site even after oral administration and would act as a depot for the slow and sustained release of Au(I) ions required for therapeutic action.

Conclusions

Colloidal metallic gold (average particle size 27 nm) is a far more potent and effective anti-arthritic agent in rats than the sodium aurothiomalate (**I**) used to treat rheumatoid arthritis. The reported data show that Au^0 administered subcutaneously (s.c.) suppressed development of three different forms of arthritis in rats. By contrast, s.c. aurothiomalate (**1**) at almost 10^3 times as gold dose was effective only against the arthritis induced with a mycobacterial adjuvant. This is the most inflammatory of these three forms of arthritis and characterised by active leukocytes generating reactive oxygen-derived species *e.g.* H_2O_2, OCl^-. Consequently, there may be more drug oxidation *e.g.* Au-SR→Au-S(O)R, thereby liberating Au(I) for dismutation to more pharmaco-active gold species, namely Au^0 and Au(III).

Swarna bhasma, as used in Ayurvedic medicine, comprises gold-containing particles that are globular and have an average size of 56-57 nm.

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