diversity exists at three levels: genes, species and ecosystems. These form a continuum and can be integrated into a hierarchical zoom. In selected cases there is also a need to understand diversity in such a context using simple but effective techniques like AFLP (amplified fragment length polymorphism), RFLP, RAPD, etc.

Lastly, the purpose of writing about mega-wildlife in *Current Science* is to bring problems underlying this group of animals to the attention of professional conservation biologists, geneticists and molecular biologists of the country. The reason is that solutions to these problems lie well beyond the traditional wildlife practice.


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**SCIENTIFIC CORRESPONDENCE**

**Photochemical formation of gold nanoparticles in aqueous Triton X-100 and its application in SERS spectroscopy**

Synthesis of gold nanoparticles is a challenging field because these crystallites have many uses. It has been used for staining proteins electrotransferred onto nitrocellulose and also as a medium for surface enhanced Raman scattering (SERS). Colloidal gold, labelled to various biological materials like lectins, antibodies, antigens, enzymes, or lipoproteins enable these systems to be observed by transmission or scanning electron microscope. It can also show varying chemical reactivity which strongly depends on the particle size. Gold sols also find use in the analytical chemistry for the determination of various polluting substances. The gold sols are characterized by an attractive colour which can vary from ruby red through purple to blue depending on the size.

Gold hydrosol, containing particles of uniform size and regular spherical shape, is usually prepared by reducing HAuCl₄ with a solution of citric acid or trisodium citrate. The method due to Moeremans *et al.* is relatively troublesome and requires boiling and refluxing. The method due to Yamaguchi *et al.* uses formalin but needs several hours stirring under appropriate pH. For maturation, it needed an additional vigorous overnight stirring. Generation of gold sol from gold(III) complex using other reducing agents such as ascorbic acid and hydrazine was also possible but proper conditions were required. Poly(ethylene glycol) and poly(vinyl alcohol) were also recently used for gold sol preparation but it took a long time.

Light-induced reactions could afford an ideal situation for many reactions. For nanocrystalline formation also, photochemical reduction could be an ideal alternative. It has important advantages in comparison to the chemical reduction. Being homogeneous, it is reproducible and can be applied at ambient temperature. Photoinitiated reduction of gold(III) complexes has recently been accomplished with acetone and 2-propanol. Here, acetone served as the sensitizer to absorb the UV light to initiate radical formation via the acetone triplet and subsequent hydrogen atom abstraction from 2-propanol. This type of photoreaction of phenols or alcohols is known in synthetic organic chemistry but is rarely applied for gold cluster preparation. Methanol also can serve as a reducing agent for gold sol preparation in a photochemical way.

Prevention of coagulation of small metal particles to form larger particles is another important criterion for nanoparticle generation. Chemically prepared metal colloids are only stable in solution, as they are protected by solvent molecules and electric charges, preventing coagulation. Addition of macro molecules like poly(vinyl alcohol) and poly(vinyl pyrrolidone), co-polymers of vinyl alcohol and N-vinyl pyrrolidone, cyclodextrins, colloidal silic acid, diallyl dimethyl ammonium chloride, sodium polyphosphate, etc. also worked well for this purpose.

Here we report a photochemical method for gold sol preparation using Triton X-100 (TX-100) in aqueous medium in a simple and quick way. Figure 1 shows the surface plasmon absorbance band (523 nm) of the zerovalent gold prepared by photolysis of the 2 ml TX-100 (10⁻² M) containing HAuCl₄ (150 ppm final concentration) for 20 min. More concentrated solutions of gold(III) can be used to obtain higher yields of gold particles. The irradiation was done with a germicidal lamp (15 W, Sankyo Denki, Japan). The samples were taken in well-stoppered 1-cm quartz cuvettes and were kept at a distance of 3 cm from the light source. Deoxygenation of the solution followed by irradiation for 20 min did not cause any change in the absorbance at 523 nm. The sol is stable for more than a month. Without TX-100, gold(III)

![Figure 1. VIS spectra of gold hydrosol after 20 min irradiation.](figure1.png)
failed to give any colour for gold sol even after 1 h irradiation. If kept in the dark (i.e. without light), aqueous TX-100 failed to generate any colour even in 8 h. A kinetic study was made to see the effect of time of irradiation on the sol production. It was found that 12–35 min time was optimum. Since TX-100 acts here both as a reducing agent and particle stabilizer, the effect of TX-100 concentration was also studied and the effective concentration was found to range from $10^{-1}$ to $10^{-3}$ M.

The size of the particles was measured using both transmission electron microscope (TEM) and Coulter (N4) counter using light scattering through an angle of 90°. Figure 2 shows the electron micrographs of the sol. It is remarkable to see the non-spherical nature of the gold particles which is rather abnormal. The average particle size observed was 14 nm.

The gold sol produced has remarkable stability towards laser illumination. It shows highly intense reproducible SERS spectrum for pyridine. Figure 3 shows an SERS spectrum of pyridine ($8.8 \times 10^{-5}$ M), obtained (a Kr laser with 100 mW power was used) after 10 min of addition of pyridine into the gold sol. The peaks observed are characteristic for pyridine molecule$^{2}$,4. On adding pyridine to the sol, a change of colour from red to blue due to aggregation is observed which is a criterion to see the SERS. This type of colour change also occurs for the gold sol prepared by the citrate method. The laser-exposed sol (with or without pyridine) can be kept under ambient condition for >7 days without any change. The spectrum was reproducible even after seven days.

Identification of the product in the process was attempted through IR spectral studies. The appearance of a new band at 1613 cm$^{-1}$ indicated the formation of carboxylate group during the photo-oxidation of TX-100 by gold(I). However, more exhaustive studies are warranted to have a clear insight into the complete mechanism.

The method for gold sol preparation described here is simple, quick and reproducible. We believe that this will offer considerable opportunities in the field of SERS and other nanoparticle research.

12. Yi, K. C., Mendela, S. M., Castañes,
Biosafety of transgenic crops: Precautions for case-by-case risk assessment

As contributors to commercial exploitation of rDNA technology in agriculture, transgenic crops were a hope a decade ago. Today, they are a reality. The commercial cultivation of a respectable number and many more that are currently being field tested for biosafety and environmental clearance stands testimony to their relevance. In spite of this and the very vigorous promotion of transgenics by proponents of the technology, concerns about potential negative impact of the transgenics on the health and nutrition of the consuming public and on the environment persist. The debate on adequacy of current procedures of biosafety testing and environmental risk assessment has become so intense as to create a vertical divide between the protagonists and the opponents. The former argue that testing of the product should not be linked to the technology used for its production and be pragmatically based while the latter advocate that only genetically engineered plants are produced by a new technology, many consequences of which have not been fully tested, should not be equated with products of conventional breeding and the projected negative effects should be proved to be absent by experimentation.

Crops differ markedly in their requirement of various components of ambient environment for optimum growth and yield, modes of reproduction and agro- nomic husbandry. When variables like nature of transgene and its source; the characteristics of transgene product and the possibility to its modification by interaction with products of other genes and the environment; flow of the transgene into other crops, crop-related wild species and non-target organisms; the nutritional, health-related effects of the transgene product, etc. are superimposed on diversity of crops, a bewildering variety of complexities confront us while determining biosafety and environmental risk assessment. No universally applicable guidelines are possible in such a situation for objective and acceptable risk assessment. For this reason, it has been accepted that the assessment should be made case by case.

According to the current procedures in countries where transgenics have graduated to commercial reality, the regulatory authorities typically require information on the following for granting permission: Large open field tests and determining biosafety and environmental risk assessment for commercial release: Donor of genetic material, Recipient. Vector or vector agent used for gene transfer, Field trial plans (test site, experimental design, containment, etc.), Product of transgene (expression, toxicity, allergenicity, pleiotropic effects, product degradation, etc.), Environmental fate (gene escape, weediness, genetic diversity, effect on non-target organisms). It is thus seen that concern is focused on the crop–transgene combination only. No account is taken of the possible contiguous cultivation of different cultivars of a crop containing the same transgene. This we believe is a serious lacuna, the relevance of which assumes even greater significance in a country like ours where farm size holdings are very small and difference among farmers for new technology absorption and practice is wide. For this reason the nature and details of case-by-case assessment in our situation will have to be different from that obtainable in the developed world. Let us illustrate this by a few examples.

First, a transgene mobilized into different crops that constitute components of a farming system. A gene for herbicide tolerance is moved into three or four crops that traditionally go into a rotation cycle. Self-sows will inevitably contribute seed to the succeeding crops. Even if we do not go by the scientific definition of what is a weed, a sizable amount of red seeds can considerably diminish the commercial value of the crop produce. The second case where the same or closely homologous transgenics are moved into the same crop, the theoretical consequences can be even more serious. It is known that multiple copies of transgene or a combination of closely homologous transgenics can result in gene silencing. It is also known that gene flow among genotypes of a crop cultivar is sizable. When different entrepreneurs and public sector institutions produce transgenics using a particular or similar gene for producing cultivars of the same crop, the fields will be mosaics of varieties containing the same gene. In such cases, following gene exchanges among cultivars, the copy number of transgenics will increase in progeny generation. If such seeds are used to raise crops, gene