Melamine modified gold nanoprobe for *"on spot"* colorimetric recognition of clonazepam from biological specimens

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Animals and Drug Administration

The animals used for investigation were housed at the Animal Care Facility, Department of Zoology, Gujarat University. The Experimental Animals: Adult male Wistar rats (Rattusnorvegicus) and Albino mice (Musmusculus) of Swiss strain, weighing between 250-350 gm & 25-45 gm respectively were procured from Zydus Cadila Health Care, Ahmedabad under the Animal Maintenance and Registration No. 167/1999/CPCSEA from the Ministry of Social Justice and Empowerment, Govt. of India. Upon arrival at the facility, the animals were given 7-15 days to acclimatize to their conditions. The animals were housed under standard temperature ($26\pm2^{o}C$), operating on a 12 h dark/light condition and relative humidity of 30-70%. Animals of experiment were caged separately and maximum of two rats per cage and five mice per cage were maintained on a standard animal food obtained from Pranav Agro Industries, containing wheat - 70%, gram - 20%, fish meat - 5% and yeast powder - 5% and distilled water was given ad libitum. Adult Mice and adult rats received oral dose of clonazepam in water at the maximum tolerated acute and chronic doses. Adult male Wistar rats (Rattusnorvegicus) and Albino mice were given acute dose of clonazepam as per the weight and sacrificed under light ether anaesthesia. The delay periods between drug administration and killing were chosen on the basis of the half-life of clonazepam as per respective animal and its weight. For chronic dose, adult Wistar rats (Rattusnorvegicus) and Albino mice (Musmusculus) received the clonazepam dose on the basis of their weight for 7 days in increasing order started with half dose of LD50 and then scarified animal buried in soil.

Blood Collection

Blood collected through the jugular vein after the 2 h of drug administration of acute dose of clonazepam and blood were kept in sterile container at low temperature.

Marrow Preparation

The bone marrows were isolated from the bone of the drug administrated animals after the formation of skeletal remain of animal body. Then it weighed 1g and dissolved in 5 mL 0.225 M NaOH with ultra-sonication.

Bone Preparation

After the transformation of animal body to skeletal remains the remaining bone samples were collected. To remove the tissues and other soil debris adhering to the surface of the bones, it was socked under hot alkaline water (0.1 M NaOH around 90^0C) for 2 h and then it scraped with a scalpel.

Extraction from blood

100 µL of blood was mixed with 200 µL of deionized water, followed by 200 µL of aqueous 0.1 mol/L zinc sulphate solution, and finally 500 µL methanol. The mixture was vortex-mixed for 1 min and kept at room temperature for 15 min. Resultant solution was then centrifuged for 5 min. After centrifugation, collected supernatant were used for further analysis.

Extraction from marrow:

Added saturated NaOH to maintain the pH range between 8–9 pH and extracted with 20ml of CH_2Cl_2-isopropanol(9:1 v/v). The organic phase was filtered through a dry sodium sulphate washed with organic phase. The solvent was immediately evaporated to dryness under stream of N_2. Bone marrow contains high amount of lipids and fat that may interfere in the process. To prevail over this problem it is re-extracted with 5ml of 0.5M HCl. The organic phase was discarded and the aqueous phase was maintained between 8.5-9pH and it was then extracted with same solvent mixture and evaporated to dryness.

Extraction from Bone:

The bone pieces were cleaned from muscle tissues and grounded in mortar. 10ml of 2M HNO_3 was added to 1g of bone powder and mixtures were demineralized at room temperature for overnight. The drug present in the bone sample was then extracted by following the procedure described above by maintaining the pH between 8.5-9.0.

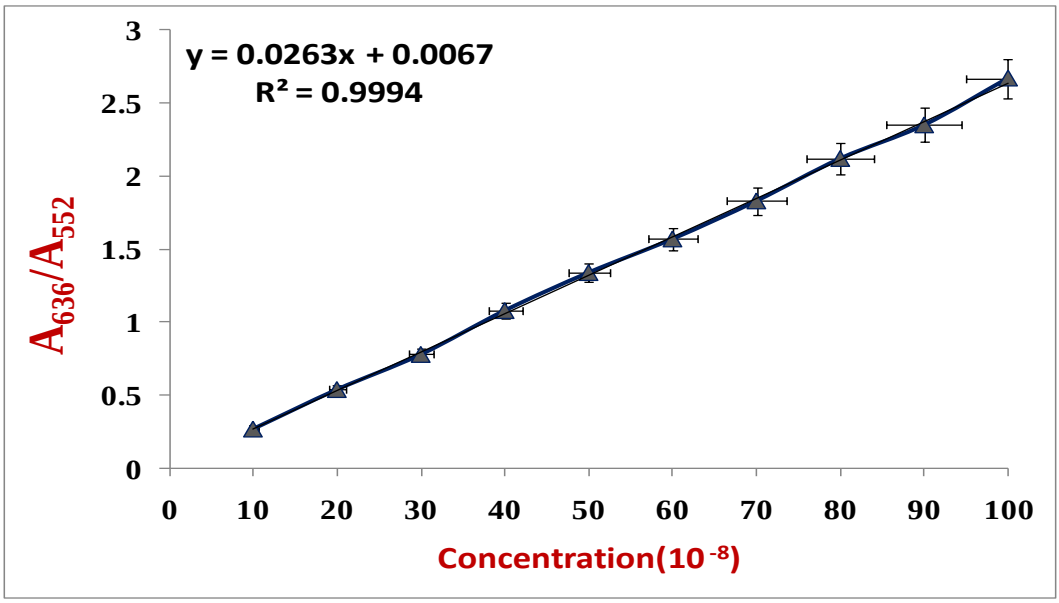

Fig.S1 Linear correlation between absorbance and clonazepam

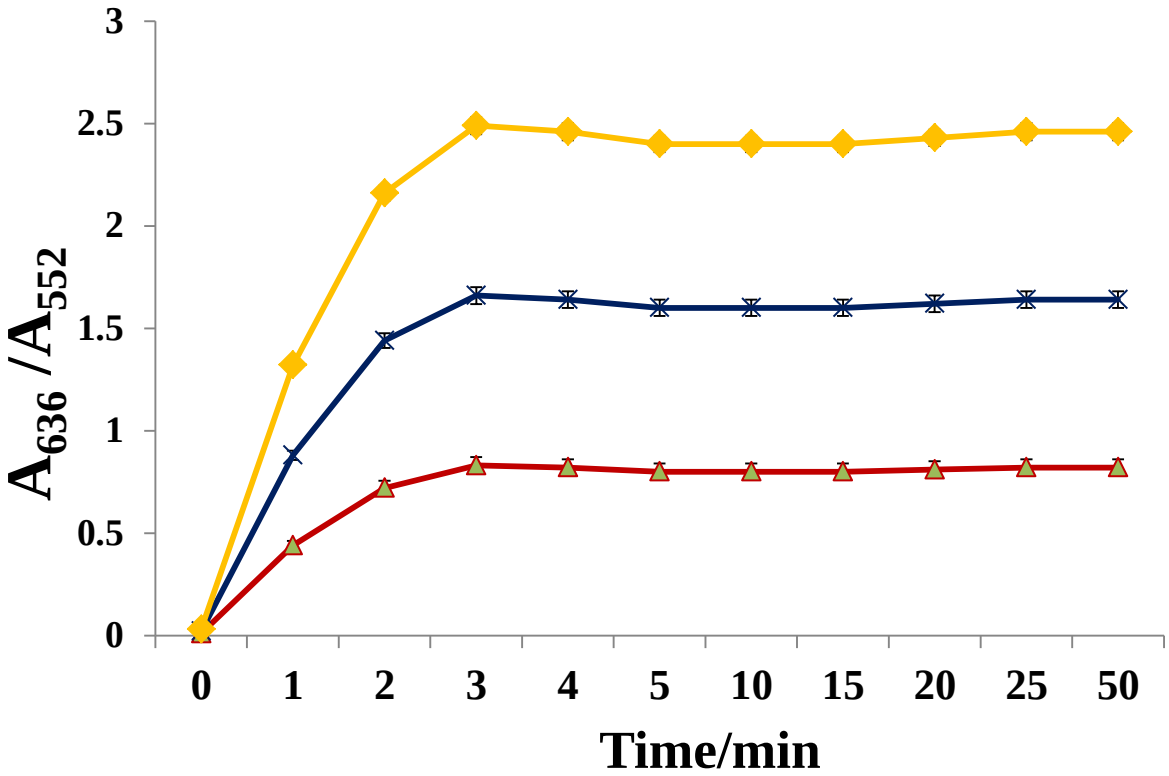

Fig.S2 The absorption ratio A_{636}/A_{552}profiles for different concentrations of clonazepam in 0.01 M PBS (pH7) with 6.4×10^{-7} M melamine.

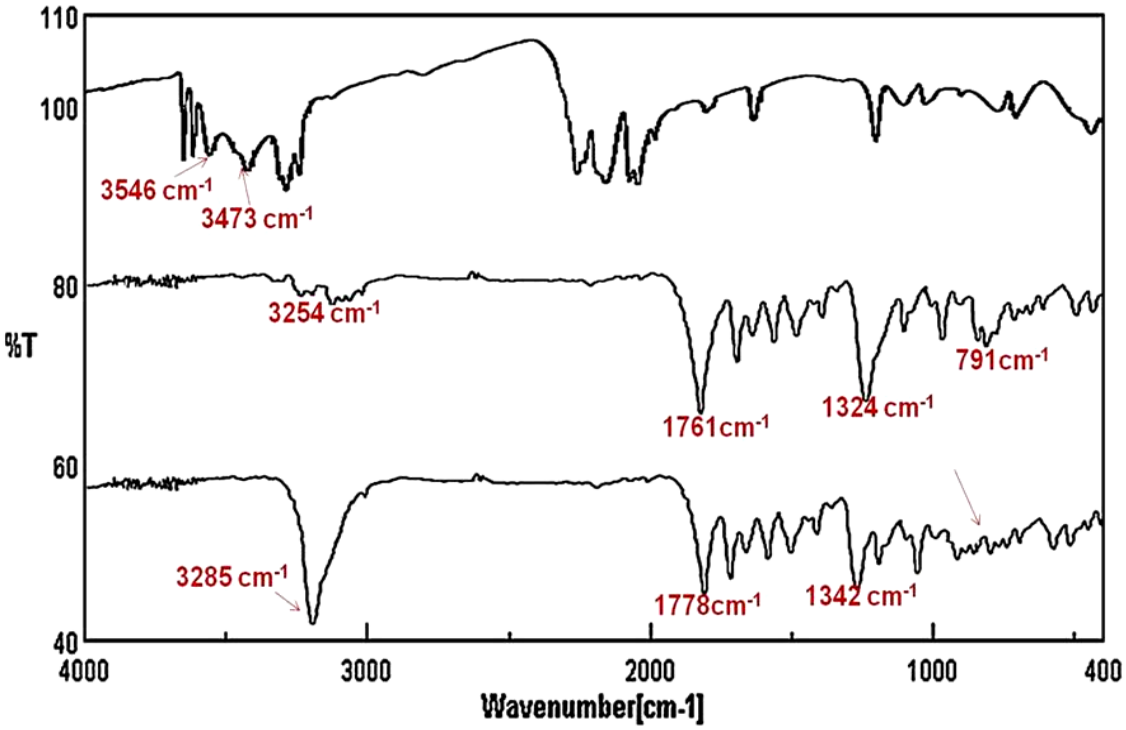

Fig S3. IR spectra of melamine (A), Clonazepam (B) and the complex of melamine and Clonazepam (C).

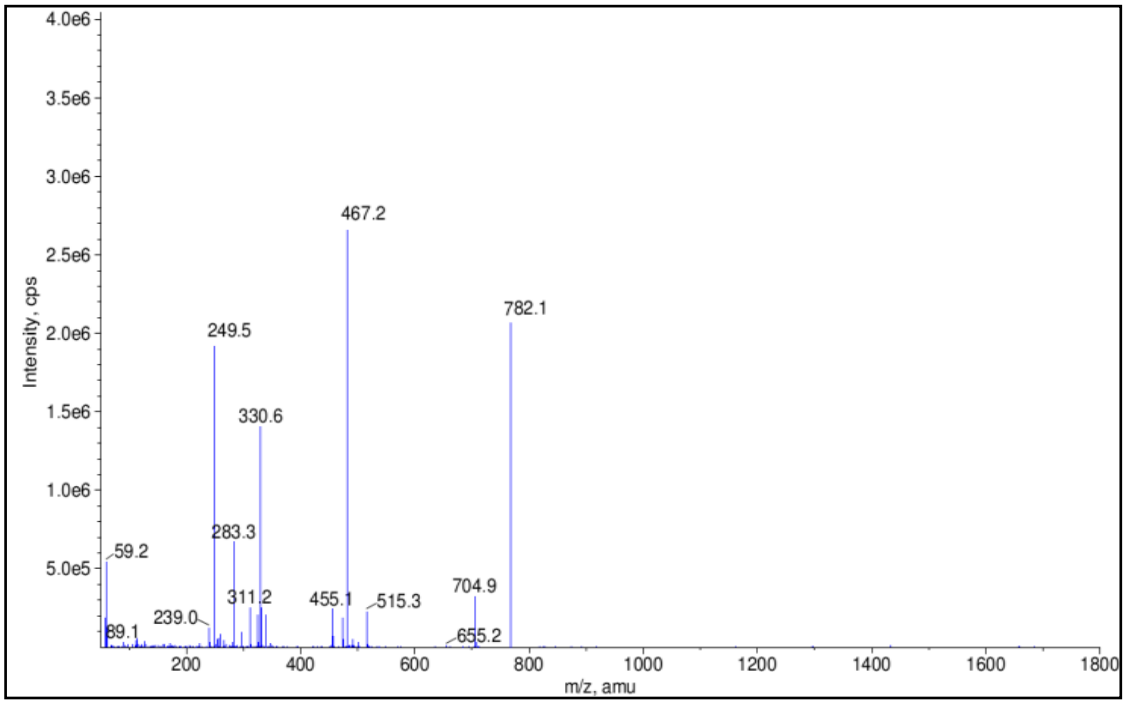

Fig. S4 ESI Mass spectra of melamine capped AuNPs and after addition of clonazepam.

Solution of pH	**4**	**6**	**8**	**10**
Stability of AuNPs	6h	Weeks	Weeks	Weeks

Table.S1 Stability of melamine modified AuNPs at different pH conditions

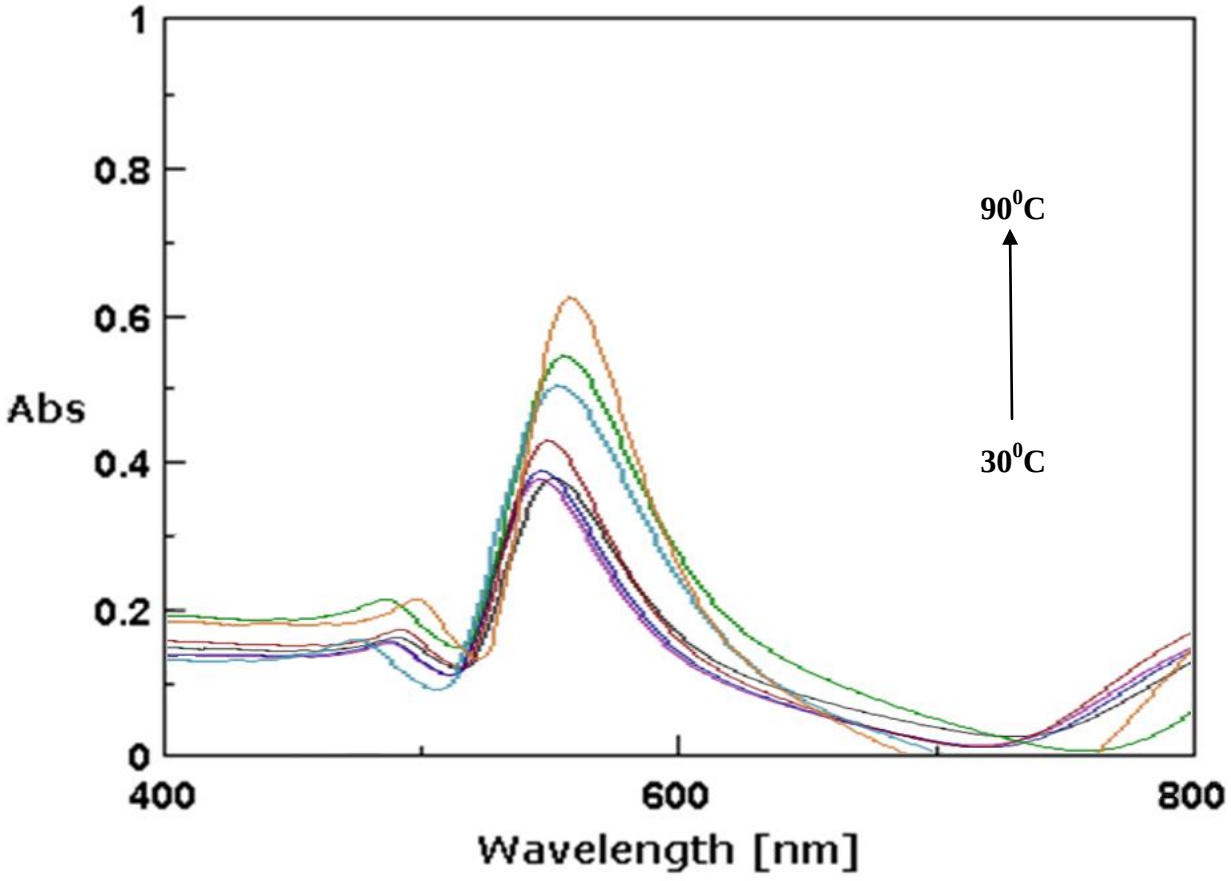

Fig. S5 The UV-Vis spectra of melamine capped AuNp of different 30^0C, 40^0C, 50^0C, 60^0C, 70^0C, 80^0C and 90^0C temperature