We confirmed that the porous PVA dried gel with GNPs embedded in the network, which can be prepared very conveniently, is a stable and ultrasensitive 3D SERS substrate. The existence of the micropores within the gel is necessary for the ultrahigh sensitivity, and embedding of GNPs in the penetrable gel results in high stability.

Surface Enhanced Raman Scattering (SERS) spectroscopy has been regarded as one of the most promising analytical techniques for detection of chemical and biological substances at low concentrations. To a great extent, the detection limit depends on the substrate used for SERS analysis. Conventional SERS-active substrates such as aggregated metallic colloids and roughened electrodes are difficult to extend to broad applications because of their low stability and/or low sensitivity. Progress has been made in designing and preparing effective SERS substrates. Among them the newly emerged 3D porous structures loaded with Au or Ag nanoparticles are especially notable since they have both high specific surface area and open morphology. The high specific surface area is useful for binding more target analytes and forming more hot spots within the laser-illumination area, and the open porous structure could provide enhanced light interactions, which can lead to great Raman enhancements.

For fabricating 3D porous SERS substrates, porous materials like colloidal crystals, porous silicon, alumina, GaN or carbon are often used as 3D templates. Various methods such as thermal decomposition, vacuum evaporation, immersion plating and electrophoretic deposition have been applied to deposit Au or Ag nanoparticles into the nano-voids within the templates; the templates loaded with the metallic nanoparticles are the 3D porous SERS substrates. The results demonstrate that most of the 3D porous SERS substrates have relatively high SERS sensitivities. However, a part of the pores were inevitably blocked during the deposition, which decreased the sensitivity. Besides, the surfaces of the metallic nanoparticles in these substrates are exposed and facilely contaminated by the environment during storing, the substrates are unstable. Obviously, a cost-effective method that can be used to fabricate SERS substrates with ever higher sensitivity and high stability is desirable.

Herein we report an ultrasensitive and stable 3D SERS substrate composed of a highly porous poly(vinyl alcohol) (PVA) dried gel with gold nanoparticles (GNPs) embedded in the network of the gel (denoted hereafter PVA–GNPs). PVA–GNPs are composed of both micro- and nano-sized pores, in which GNPs were embedded within the network that is swollenable and thus penetrable by small molecular solutes. When crystal violet (CV) in methanol was used as the probe molecule, the detection limit of CV reaches as low as $10^{-12}$ M. In addition, the same detection limit can be achieved after storing the PVA–GNPs substrate for 3 months. We confirmed that the existence of the micropores within the gel, which was thought to be unimportant for Raman enhancement in the existing studies, is necessary for the ultrahigh sensitivity, and embedding of GNPs in the penetrable network results in high stability.

GNPs used in the present study were synthesized according to a citrate reduction method reported previously. The as-prepared GNPs show a narrow size distribution with an average diameter of 45 nm (Fig. 1A). The PVA–GNPs were simply fabricated by gelation of PVA in H$_2$O–DMSO ($V/V = 1/4$) in the presence of the GNPs and micron-sized NaCl particles (as pore-forming agents), dialysis of the gel to remove NaCl and freeze-drying of the gel (see S1 in ESI†). As shown in Fig. 2A and B, the resultant PVA–GNPs are highly porous. Most of the pores are several microns in size, close to the size of the pore-forming agent (Fig. 2C). Besides, there are also nano-sized pores surrounding the micropores, as indicated by the red arrows in

![Fig. 1](A) TEM image of GNPs. (B) UV-Vis absorption spectra of gold colloidal solution and porous PVA–GNP gel (before freeze-drying).
Fig. 2B, which should result from solvent sublimation during the freeze-drying process. The dispersion of GNPs within the gel was characterized by TEM observations (Fig. 2D) of ultrathin sections of the PVA–GNP porous gel (before freeze-drying). It was found that the majority of GNPs are embedded in and thus encapsulated by the network of the gel (Fig. 2D and Fig. S2, ESI†).

TEM observations of the ultrathin sections of PVA–GNPs also demonstrate that some GNPs aggregate into gold dimers or clusters within the network (please see the insets in Fig. 2D), which is further evidenced by the UV-Vis measurements. As shown in Fig. 1B, the UV-Vis spectrum of the GNPs individually dispersed in the solution shows a single and strong absorption band peaked at 529 nm, whereas that of the gel displays not only a wide plasmon band at around 531 nm but also a weak signal above 700 nm; the UV-Vis spectrum of PVA–GNPs reveals aggregation of the GNPs within the network.17 Both the aggregation of GNPs and the abovementioned bimodally porous structure are advantageous for the excellent SERS performance of the porous PVA–GNP gel.6

To evaluate the SERS performance of the PVA–GNP substrate, crystal violet (CV) was used as the model probe.18 For the measurement, a slice of PVA–GNPs of about 2 mg weight was immersed in 1.0 mL of the methanol solution of CV for 24 h. Then, the gel slice was taken out and dried under ambient conditions and subsequently subjected to Raman analysis. The SERS spectrum of the substrate was treated with the CV solution at $10^{-9}$ M and the Raman spectrum of the pure substrate are shown in Fig. 3 as spectra a and b, respectively. Strong Raman signals of CV are observed in the spectrum a (the assignment of the peaks is given in Table 1); the substrate enhances the Raman signals greatly because CV at a concentration lower than $10^{-3}$ M cannot be detected without the assistance of a SERS substrate.18 Besides, the substrate has a clean background, since the spectrum of the pure substrate (spectrum b) measured under the same conditions as spectrum a shows no any signals. This provides the precondition for ultrasensitive Raman detection.

The detection limit of CV on the substrate was determined by measuring SERS spectra of CV at the varied concentrations from $10^{-7}$ to $10^{-13}$ M. As shown in Fig. 4, when the concentration of CV is larger than or equal to $10^{-12}$ M (spectra a–f), SERS signals of CV can be clearly observed. Therefore, the detection limit is as low as $10^{-12}$ M, which is lower than all of the detection limits previously reported based on Au nanoparticles.

For investigation of the effect of the 3D porous structure on SERS sensitivity, a PVA–GNP cast film with the same composition but without pores (Fig. 5A) was used for SERS analysis of CV. The detection limit of CV was determined to be $10^{-7}$ M (Fig. 6), which is much higher than $10^{-12}$ M (the detection limit of CV on the PVA–GNP porous gel). In the two systems, the GNP/gel weight ratios are the same and the GNP–GNP and GNP–PVA interactions are similar. Thus, the distribution and aggregation state of GNPs in the two substrates should not be remarkably different. The much higher sensitivity of the PVA–GNP porous gel should mainly result from the 3D porous structure. It should be mentioned here that, in the present study, for determination of the detection limit of CV on a substrate, at least 20 points randomly selected on the substrate were measured, and the spectrum with the best sensitivity was used. Therefore, the difference in the detection limit arising from the sampling can be avoided (see S2 in ESI†).
As mentioned before, the PVA–GNP porous gel (denoted as Gel I hereafter) has bimodal pores, and the micropores mainly result from the presence of the pore-forming agent during the gelation. The great effects of nanopores within 3D porous substrates on the Raman enhancements have been reported, while micropores in the substrates were thought to be unimportant for the enhancements. However, we confirmed that the micropores are indispensable for Gel I to act as an ultrasensitive SERS substrate. This is exhibited by the control experiment using the PVA–GNP gel (Gel II) that was prepared under the same conditions but without the pore-forming agent as the SERS substrate. Gel II is still highly porous but most of the pores are nano-sized (Fig. 5B). When Gel II is used for SERS analysis, the detection limit of CV is about 10^{-8} M (Fig. 6 and Fig. S4 and S5 in ESI†), which is much higher than that achieved using Gel I (10^{-12} M). A possible mechanism for the effect of micropores on SERS sensitivity is given in S6 in ESI†.

The stability of SERS substrates is very important for the use of the SERS technique as a routine analytical tool. To investigate its storing stability, Gel I was stored under ambient conditions for 3 months and then used for measuring the detection limit of CV. It is significant that the same detection limit was achieved after the relatively long storing time (Fig. 7); the substrate is highly stable. The high stability is quite reasonable since most of the GNPs are encapsulated in the network of the gel, so that the surface of GNPs and their aggregation state are well protected. Besides, the encapsulation does not affect the accessibility of GNPs to CV, since the PVA gel is penetrable by small molecules according to Yang J. M. et al.

In conclusion, we have demonstrated a new 3D porous SERS substrate (PVA–GNPs) with ultrahigh sensitivity, on which the detection limit of CV is as low as 10^{-12} M. The substrate is also highly stable since the detection limit of CV on the substrate stored under ambient conditions for 3 months is still 10^{-12} M. Besides its excellent SERS performance, the preparation processes for the substrate are very convenient and cost-effective. We believe that the substrate is very promising for routine measurements of small organic analysts. The great effect of the bimodal pores on the sensitivity of the SERS substrate should be inspiring to the efforts to search for ultrasensitive SERS substrates.

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Notes and references