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Less chemicals for more controlled syntheses: on the benefits of mixtures of alcohols for room temperature surfactant-free colloidal syntheses of gold nanoparticles†

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The full exploitation of nanoparticle (NP) properties requires their controlled syntheses, ideally achieved by more sustainable strategies. We report on the benefits of using mixtures of ethanol and glycerol for the room temperature syntheses of stable surfactant-free colloidal gold (Au) NPs in alkaline water–alcohol solutions. Using [ethanol + glycerol] mixtures with a low amount of glycerol, e.g. only 2 v%, improves the size control over the Au NPs and enables the total alcohol content to be lowered for a successful synthesis. The results provide solid foundation towards the rational development of more sustainable syntheses of colloidal NPs.

Nanoparticles

Nanomaterials and nanoparticles (NPs), e.g. gold (Au) NPs, exhibit unique properties that are relevant for applications in multiple areas of research and development, e.g. in catalysis, sensing, optics, electronics or medicine.^{1,2} Regardless of the application considered, the full exploitation of NP properties requires their controlled syntheses.^{3–5} The synthesis would ideally be simple, safe, widely implementable and scalable. Various methods have been reported for the preparation of Au NPs.^{1,6} Typically, Au NPs are obtained using a wide range of chemicals playing multiple roles.⁷ The rational selection of the chemicals can therefore be tedious. Moreover, several of those chemicals can be harmful. There is therefore an increasing awareness and need for production of NPs by greener and more sustainable methods.^{8,9}

Sustainable syntheses

Towards the development of more sustainable syntheses, so-called *surfactant-free* (SF) colloidal syntheses are promising

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Sustainability spotlight

Nanotechnologies can address several of the UN's Sustainable Development Goals (SDGs), provided *sustainable* synthesis methods of nanomaterials are proposed. Nanomaterials syntheses are typically developed by waste-generating trial-and-error selections of often-harmful chemicals. Here, with the example of gold nanoparticles, the rational selection and mixtures of benign alcohols allows the sustainable synthesis of colloidal nanoparticles (i) with controlled nanoparticle formation leading to controlled sizes and (ii) using a minimal total amount of chemicals. The concept aligns with UN SDGs: industry, innovation and infrastructure (SGD 9). Given the wide range of applications of nanomaterials, and gold nanoparticles in particular, the findings are relevant for optics, electronics, catalysis, energy conversion, water/air treatment, sensing and/or medicine and many more to best address the *Green Transition*.

strategies. In these approaches, no additives with a molar mass higher than 100 g mol^{−1} apart from the metal precursor are required, as per the definition proposed and detailed elsewhere.^{10,11} Since in those approaches size and/or shape control cannot be achieved by the typical use of common additives such as citrates or thiols,^{7,12} new strategies need to be developed.

In particular, the use of a mixture of water and an alcohol under alkaline conditions is promising to simply lead at room temperature (RT) to stable SF colloidal Au NPs.^{13–17} Possible knobs previously investigated to achieve size control in this approach are different concentrations of HAuCl₄ and/or base, or different alcohol contents.^{16,18,19} Recently, a comparison of different alcohols as the source of reducing agents shed light on a promising yet scarcely explored alternative option to achieve size control by using different alcohols.²⁰ An account of the pros and cons of using different alcohols is proposed in Table S1† and detailed below.

Polyols

To date, a strong – and almost exclusive – focus has been given to glycerol for such RT SF colloidal synthesis of Au NPs.¹⁶ The



glycerol-mediated synthesis is fast and lead to relatively small size NPs in the range 5–10 nm under a wide range of conditions, *e.g.* different concentrations of base and/or alcohol contents.²⁰ The synthesis using glycerol is also less sensitive to the light environment. The glycerol-mediated synthesis is therefore overall more *robust*, which probably accounts for its popularity to date. However, a major drawback of the glycerol-mediated synthesis is the use of a relatively viscous solvent. The high viscosity of polyols prevents a simple and direct use of the Au NPs without extensive and waste-generating washes.^{21,22} Ethylene glycol is a possible alternative to glycerol,²³ but is still a viscous solvent with similar pros and cons.²¹

Mono-alcohols

Recently, methanol and ethanol were reported as possible alternative candidates. The RT synthesis can be performed using *ca.* 20 v% of mono-alcohols as the source of reducing agents.^{24,25} The method detailed elsewhere^{18,26} leads to size-controlled NPs in the range 5–30 nm. Despite the absence of common additives, the method leads to colloidal dispersions of NPs stable for years.²⁷ The method presents the benefits of requiring cheap, relatively low toxicity and low viscosity chemicals, which are desirable features to more easily process the NPs, *e.g.* to deposit the NPs on various surfaces to develop heterogeneous catalysts without the need for intensive and waste-generating steps that typically require strong acids when polyols are used.^{3,21} Nevertheless, the overall kinetics of NP formation are slower and the experimental window where the synthesis can be considered successful is narrower than in the case of polyol-mediated syntheses.²⁰ The use of methanol is not preferred due to the higher toxicity of methanol²⁸ and the fact that methanol-mediated syntheses lead to larger *ca.* 10–30 nm NPs.

Mixtures of polyols and mono-alcohols

The rational selection of complex solvent mixtures and/or combination of the right reducing agents to perform NP syntheses is generally a tedious task.²⁵ Exploring solvent mixtures with three or more chemicals is rarely a preferred option due to the inherent complexity of the approach in the selection process of those solvents and their relative ratios. The different pros and cons of polyols *versus* mono-alcohols have just recently been clarified.²⁰ With this knowledge at hand, we here explore the potential benefits of mixtures of alcohols.

Hypothesis

A driving hypothesis here is that the formation of the Au NPs is sensitive to the conditions under which the very initial steps of the synthesis take place. This hypothesis is motivated by the findings from a previous study focusing on different light environments, where a hybrid approach of light-controlled environment and dark conditions shows that the light conditions have a strong influence mainly at the beginning of the synthesis. The use of lights with low wavelengths to obtain small size NPs was mainly key in the first minutes of the reaction.²⁰

To the best of our knowledge, the strategy to use [ethanol + glycerol] mixtures to optimize SF syntheses of Au NPs has not been reported before. It is here hypothesized that a small amount of glycerol helps to achieve size control towards smaller NPs by playing a role in the initial steps of the synthesis by favouring a faster formation of the NPs. Therefore, we explore for the first time, to the best our knowledge, the possible benefits of using [mono-alcohol + polyols] mixtures as the source of reducing agents for the RT SF colloidal syntheses of Au NPs, with a focus on high mono-alcohol content.

Methods

A detailed account of the synthetic procedures, characterization methods used and related metrics is proposed in ESI, see Scheme S1, Fig. S1 and Table S2.† As much as possible the experimental design follows the principles of *Sustainability in the chemistry laboratory*.²⁹

Results

It is here considered that desirable features for a synthesis, and yet challenging features to combine, are to lead to small size NPs, in a relatively timely approach, through a robust process and ideally in low viscosity solvents to facilitate the use and processing of the Au NPs.^{16,21} An overview of the samples discussed and the results obtained using different amounts of ethanol, glycerol and [ethanol + glycerol] mixtures are summarized in Table S3.† UV-vis spectra are reported in Fig. S2† with further analysis in Fig. 2 and S3–S7.† The corresponding STEM characterization and size distributions are provided in Fig. S8 and S9.†

Proof of concept

Upon adding H₂AuCl₄ to an alkaline solution of 80 v% water and 20 v% alcohol the solution turns grey, blue, purple and finally red, indicative of the formation of Au NPs with a characteristic surface plasmon resonance (spr) around 520 nm in UV-vis characterization. The synthesis proceeds in a similar way when methanol, ethylene glycol or glycerol (only one alcohol) are used, with however different kinetics and leading to different NP sizes, which is attributed to the different redox and physico-chemical properties of the different alcohols.²⁰ The alkaline conditions favour the formation of alkoxides from the alcohols that play the role of reducing agents for the RT process.²⁴ The general mechanisms behind the NP formation are detailed elsewhere,^{18,20,24} and further discussed below in light of the results reported. A first finding here is that Au NPs are also obtained when mixtures of [ethanol + glycerol] are used, as illustrated in Fig. 1.

Effects of the amount of ethanol

When only ethanol is used as the source of reducing agent, and when 20 v% ethanol is used, the NPs show a λ_{spr} value around 524 nm which corresponds to 13.8 ± 3.9 nm NPs. As the amount



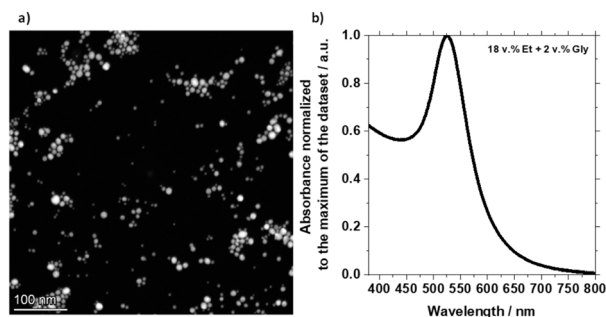


Fig. 1 (a) Illustrative STEM micrograph and (b) UV-vis spectra of Au NPs obtained using [18 v% ethanol + 2 v% glycerol]. 0.5 mM HAuCl₄ and 2 mM NaOH were used. Et: ethanol; Gly: glycerol.

of ethanol decreases to 19 or 18 v% the λ_{SPR} values remain at 524 nm and increase to 526 nm respectively, corresponding to 12.1 ± 4.5 nm and 16.5 ± 4.4 nm, respectively, see Fig. 2. The trend that the NP size increases and/or the size distribution increases when the amount of ethanol decreases is confirmed when 10 v% of ethanol is used, for which the λ_{SPR} value increases to 558 nm corresponding to 18.6 ± 12.1 nm NPs. This corresponds to a case where there is not enough reducing agent and the NP synthesis is less controlled.¹⁸ The poorer control over the synthesis is even more pronounced for 2 and 1 v% ethanol where there is not enough alcohol to perform the reduction of Au^{III}, as indicated by relatively featureless UV-vis spectra with an overall low absorption, *e.g.* very low A_{400} values compared to the other samples, see Fig. S2 and S4.† As a result, no STEM characterization was performed on those samples obtained with 1 or 2 v% ethanol.



Fig. 2 λ_{SPR} values retrieved from UV-vis characterization (\square , left-hand side axis) and NP diameter (\circ , right-hand side axis) retrieved from STEM analysis as a function of different alcohol contents and mixtures, as indicated. The source of reducing agent was ethanol only in various contents, [ethanol + glycerol] in various ratios, and glycerol only in various contents, as indicated. X indicates samples for which the plasmon resonance is not well pronounced: 1 and 2 v% ethanol. Et: ethanol; Gly: glycerol. See Fig. S2† for UV-vis spectra and Fig. S3† for further correlation between λ_{SPR} and diameter. In all cases 0.5 mM HAuCl₄ and 2 mM NaOH were used.

Effects of the amount of glycerol

With only glycerol as the source of reducing agent, using 20, 19, 18, 10, 2 or 1 v% glycerol, the λ_{SPR} values are 518, 522, 520, 522, 542 and 555 nm, respectively, and the size of the NPs are 8.0 ± 2.5 , 9.3 ± 3.4 , 8.2 ± 2.7 , 9.5 ± 2.9 , 21.2 ± 9.8 and 22.1 ± 9.7 nm, respectively, see Fig. 2. The fact that NPs are obtained even at very low amount of 2 or 1 v% illustrates the strongest reducing properties of glycerol compared to ethanol. Using 20, 19, 18 or 10 v% glycerol does not affect much the resulting size of the Au NPs which illustrates the previously documented robustness of the synthesis using glycerol, less sensitive than ethanol-mediated syntheses to the variations of experimental conditions, *e.g.* the amount of alcohol.²⁰ Interestingly, using 1–2 v% glycerol seems enough to initiate the formation of the Au NPs, although it can be expected that there is not enough reducing agent to perform the full reduction of the Au NPs and/or the reaction is much slower as detailed below, as indicated by the lower A_{400} values retrieved, see Fig. S4.† At lower amounts of alcohol, the syntheses also tend to be less reproducible. Interestingly, the sizes of the Au NPs using 20, 19, 18 or 10 v% glycerol are smaller than when 20, 19, 18 or 10 v% ethanol, respectively, are used, in agreement with previous reports.¹⁸

Effects of the amount of [ethanol + glycerol]

Having established that (i) *ca.* 20 v% ethanol is suitable to obtain relatively small size Au NPs in a low viscosity solvent and that (ii) low amounts of glycerol are suitable to induce the Au NP formation, we now turn to investigate mixtures of [ethanol + glycerol] in different relative ratios but keeping the total amount of alcohol at 20 v%, see Fig. 2. Using [19 v% glycerol + 1 v% ethanol], [18 v% glycerol + 2 v% ethanol] or [10 v% glycerol + 10 v% ethanol], leads to colloidal dispersions characterized by λ_{SPR} values of 519, 523 and 518 nm, respectively, which correspond to Au NPs with sizes of 8.5 ± 2.7 , 8.9 ± 2.9 and 7.2 ± 2.1 nm respectively. The use of only 19, 18 or 10 v% glycerol without ethanol added leads to λ_{SPR} values of 518, 522 and 520 nm, respectively, which corresponds to Au NPs with sizes of 8.0 ± 2.5 , 9.3 ± 3.4 , 8.2 ± 2.7 nm, respectively. In conclusion, there are no benefits to use [ethanol + glycerol] mixtures with high glycerol contents, given that similar NP sizes are obtained for an equivalent amount of glycerol without ethanol.

However, a different picture emerges when 2 v% of glycerol is used while the ethanol content is 18 v%. For [18 v% ethanol + 2 v% glycerol], the λ_{SPR} value is 521 nm for a size of 7.5 ± 2.8 nm. This is significantly lower than for 20, 19 or 18 v% ethanol without glycerol (λ_{SPR} values of 524, 524, 526 nm, respectively) and significantly lower than if only glycerol at 2 v% was used (λ_{SPR} value of 542 nm for 21.2 ± 9.8 nm). Using [19 v% ethanol + 1 v% glycerol] (λ_{SPR} values of 524 nm for a size of 16.7 ± 5.3 nm) did not always lead to much improvement compared to a case where 20 v% ethanol or 19 v% ethanol was used (λ_{SPR} values of 524 and 524 nm, respectively, sizes of 13.8 ± 3.9 and



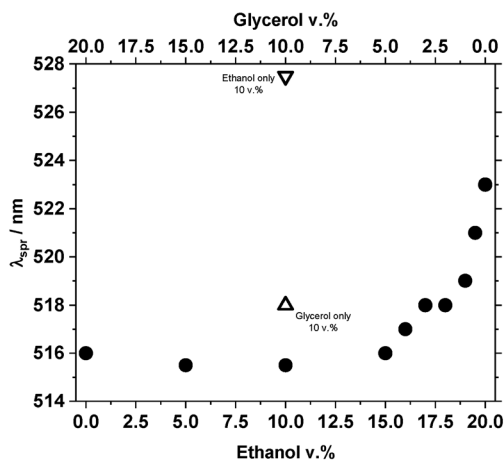


Fig. 3 λ_{spr} values retrieved from UV-vis characterization as a function of different alcohol contents and mixtures, as indicated, for repeated experiments, *i.e.* different dataset than in Fig. 2. See Fig. S3† for further correlation between λ_{spr} and diameter. Unless otherwise specified the total amount of alcohol was 20 v%, 0.5 mM HAuCl₄ and 2 mM NaOH were used in all cases.

12.1 ± 4.5, respectively). The lack of improvement in this case is attributed to the too low amount of glycerol added, as detailed in the ESI.†

A more detailed screening of the influence of low amount of glycerol is proposed in Fig. 3 together with repeated experiments that confirm the trend observed, see Fig. S5–S7 and S9.† These results indicate that the NP size decreases as the amount of glycerol increases to *ca.* 5 v%, while the viscosity of the reaction mixture increases as the glycerol content increases.¹⁶

In conclusion, small amounts of polyol in the range 1–5 v% helps controlling the NP size towards smaller sizes as the amount of glycerol increases. Importantly, this small amount of viscous polyol does not change much the overall viscosity of the samples still obtained in relatively low viscosity media containing mainly water and ethanol.

Kinetics

To understand better the effect of such a small amount of glycerol on the control of the NP size, we performed kinetics study on the formation of the Au NPs. Fig. S10† reports the results of time-resolved studies of the Au NP formation using various alcohols or alcohol mixtures, for a total amount of 20 v%. The related UV-vis spectra the day after synthesis are provided in Fig. S11.† The size of the Au NPs from experiments performed with 20 v% glycerol, [18 v% ethanol + 2 v% glycerol] and 20 v% ethanol is 8.2 ± 2.2, 7.7 ± 3.0 and 11.0 ± 4.3 nm, respectively, corresponding to λ_{spr} of 519, 518 and 528 nm, respectively. STEM micrographs and size distributions are also provided in Fig. S10.†

Further analysis of the kinetic UV-vis data is shown in Fig. 4 and S12.† The overall kinetics of formation of the Au NPs, estimated when various metrics reached a steady state, are rather slow using ethanol compared to the case where glycerol is used. This observation is in agreement with the more rapid

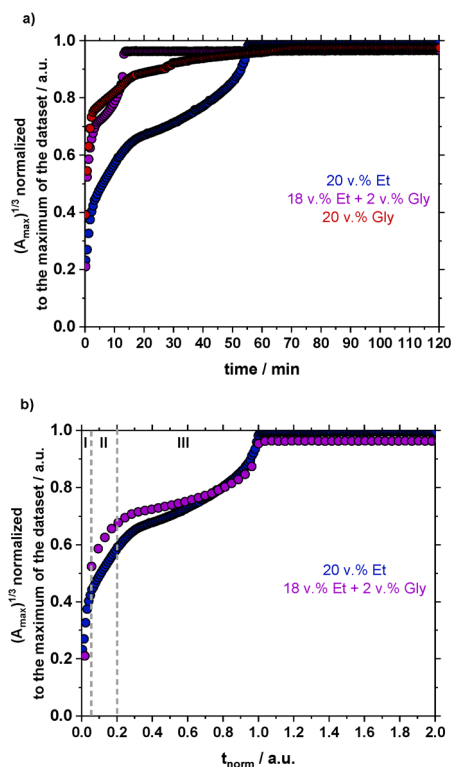


Fig. 4 (a) Normalized $(A_{\text{max}})^{1/3}$ as a function of time during the synthesis of the Au NPs as a function of different alcohol contents, as indicated. (b) Illustration for the sample obtained with 20 v% ethanol and [18 v% ethanol + 2 v% glycerol] the three different phases I–III detailed in the text, as indicated. (b) Normalized $(A_{\text{max}})^{1/3}$ plotted with the time normalized to the time where a steady state is reached. 0.5 mM HAuCl₄ and 2 mM NaOH were used in all cases. Et: ethanol; Gly: glycerol.

formation of red colloidal dispersions (which can be observed with the naked eye) when glycerol is used compared to the use of ethanol only. When [ethanol + glycerol] mixtures are used, and even with small amounts of glycerol such as 2 v% glycerol with 18 v% ethanol, an overall faster appearance of a red colloidal dispersion can be observed with the naked eye compared to the case where ethanol only is used. The overall formation pathway considering various metrics is confirmed to be faster for [ethanol + glycerol] compared to the case of 20 v% ethanol without glycerol, see Fig. 4a.

$(A_{\text{max}})^{1/3}$ is proportional to $R(N)^{1/3}$ where N is the number of particles and R their radius.³⁰ The general features of the time-resolved data resemble those reported by others, for example on study considering the citrate-mediated synthesis of Au NPs. The plot of $(A_{\text{spr}})^{1/3}$ as a function of time can be divided in to three phases:³⁰ (I) an initial region where $(A_{\text{spr}})^{1/3}$ increases in a superlinear fashion; (II) an intermediate region where $(A_{\text{spr}})^{1/3}$ increases linearly; and (III) a final region where $(A_{\text{spr}})^{1/3}$ exponentially increases and levels off to its final value. For clarity, those phases (I–III) are indicatively illustrated in Fig. 4b. Those phases have been attributed to:³⁰ (I) NP growth *via* aggregation (coupled to SAXS measurements, it was established in previous work that the number density of the NP decreases, while the particle size increases in phase I³⁰); (II) no further aggregation



occurs, and the particles grow linearly in time *via* a surface growth reaction; and (III) NP size grows *via* an autocatalytic growth stage and levels up to its final value.

Here, in agreement with previous results,²⁰ the formation of the Au NP using 20 v% glycerol (no ethanol) is rather fast in the initial steps of the synthesis, followed by overall a slower and more continuous growth, which is attributed to the redox properties of glycerol and its high viscosity, respectively. Also in agreement with previous reports for the ethanol-mediated synthesis (no glycerol), the phases I and II are almost merged which is attributed to the fast formation of the first NP seeds in the absence of stabilizers in the low viscosity solvent together with a slow autocatalytic growth due to the RT process.²⁰ Note that the focus on the present work at this stage is not to fully elucidate the formation pathway at stake here but illustrate the differences between the use of glycerol or ethanol.

In the case of [ethanol + glycerol], the same phases are observed but the reaction proceeds faster than when only ethanol is used. Importantly, the time normalized data from 20 v% ethanol and [ethanol + glycerol] with 18 v% ethanol and 2 v% glycerol follow the same general dynamics illustrated in Fig. 4b. This suggests that glycerol mainly plays a role to accelerate the initial steps of the synthesis. Since all conditions lead to approximately the same final $(A_{\text{spr}})^{1/3}$ values, and assuming that using different alcohols does not change too much the properties of the medium surrounding the Au NPs and hence their optical properties, and given the larger size of the resulting NPs when ethanol only is used, as detailed in Fig. S10,[†] it can be concluded that the number of final NPs obtained in the presence of glycerol is higher than without.

Therefore, the difference observed can be interpreted as follows: small amounts of glycerol favour a faster formation of the initial seeds. Assuming that the classical nucleation theory applies here,³¹ this fast formation of the seeds followed by a slow growth leads to the observed smaller and more mono-dispersed NPs compared to the case where only ethanol is used (where the use of ethanol only is characterized by a slower nucleation process). Ethanol plays a role by providing extra reducing agents for the further growth and/or stabilization. A direct consequence of the fastest initial formation of the Au NPs is to develop more robust syntheses, *e.g.* less sensitive to the influence of light as detailed in Section 5 in the ESL.[†] The fast formation is observed with an amount of glycerol as low as 2 v%.

Optimization towards more sustainable syntheses

The opportunity to use a small amount of glycerol finally pushed us to investigate lower total contents of alcohols. Mixtures with total amounts of *ca.* 10 v% and 5 v% were considered. For the same reasons as those detailed above, a focus was on mixtures with higher ethanol contents than glycerol. The results are reported in Fig. 5, S15 and S16.[†] Using 10 v% ethanol, 8 v% ethanol, 5 v% ethanol or 3 v% ethanol did not lead to stable colloids, no well-defined spr was observed and

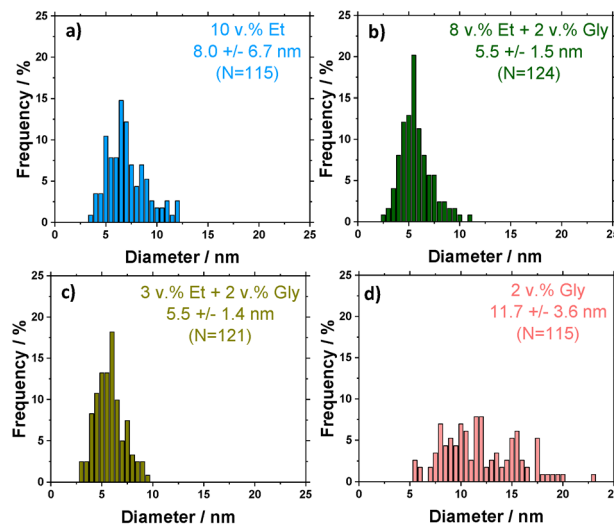


Fig. 5 Size distributions for Au NPs prepared using (a) 10 v% ethanol, (b) [8 v% ethanol + 2 v% glycerol], (c) [3 v% ethanol + 2 v% glycerol] and (d) 2 v% glycerol. Et: ethanol; Gly: glycerol. In all cases 0.5 mM HAuCl₄ and 2 mM NaOH were used. Illustrative corresponding STEM micrographs are reported in Fig. S17.[†] As per the Experimental section (see ESI[†]) the NPs were obtained using 2 hours of controlled light and then left overnight on the bench before further characterization.

the results tend to be less reproducible as the ethanol content decreases, in agreement with the results presented above. Using 2 or 3 v% glycerol also did not lead to stable colloids and the results tend to be more difficult to reproduce. However, the use of [8 v% ethanol + 2 v% glycerol] or [3 v% ethanol + 2 v% glycerol] leads to improved syntheses: more defined λ_{spr} peaks after 24 hours (compared to the case where 10 v% or 5 v% ethanol was used) and smaller λ_{spr} values than if 2 v% glycerol only was used. The Au NP colloids are also more stable over time as detailed in the next section.

The size of the NPs is improved towards smaller size when [ethanol + glycerol] mixtures with lower total amounts of alcohols are used, see Fig. 5. Using 10 v% ethanol leads to NPs with a large size distribution (8.0 ± 6.7 nm, due to the presence of very large NPs above 25 nm). Using only 2 v% glycerol leads to relatively large NPs after 24 hours (11.7 ± 3.6 nm). In contrast using [8 v% ethanol + 2 v% glycerol] leads to smaller NPs (5.5 ± 1.5 nm). The total amount of alcohol can even be reduced to 5 v% to still lead to smaller size NPs (5.5 ± 1.4 nm).

Overall the results illustrate the benefits of mixing alcohols to: (1) perform the synthesis in less viscous media; (2) develop faster and more robust synthesis; (3) optimize the total amount of alcohols towards lower values; to (4) obtain small(er) size NPs. Overall the concept of mixing alcohols as the source of reducing agents allows the development of more sustainable syntheses requiring fewer chemicals to yet lead to smaller NPs.

Stability

Despite the absence of added stabilizers, the Au NP colloids are stable for weeks as detailed in Fig. S18 and S19,[†] where the UV-



vis spectra of the as-prepared NPs and the UV-vis spectra of the same batch after 7 months of storage at RT in a drawer are almost the same (the measurements were performed without active homogenization of the sample prior to the measurement). This is line with the stability of SF NPs prepared with 20–30 v% stable for years.²⁷ Importantly, studying the samples over time, we could observe that the samples obtained with 2 v% glycerol ultimately slowly turned red, leading to Au NPs. However, those solutions were less stable over time and show higher λ_{spr} values than those obtained using [18 v% ethanol + 2 v% glycerol], [8 v% ethanol + 2 v% glycerol] or [3 v% ethanol + 2 v% glycerol], see Fig. S15–S19.†

While the stabilization mechanism(s) of the Au NPs is still to be fully established, electrostatic interactions seem key and the contribution of species such as acetaldehyde (oxidation product of ethanol) cannot be ruled out,^{32,33} given the need for glycerol and ethanol for a successful synthesis.

Conclusions

Mixtures of alcohols such as [ethanol + glycerol] mixtures can be used as the source of reducing agents for the RT synthesis of stable SF Au NPs in alkaline aqueous media. Based on evidence from UV-vis characterization and STEM microscopy, a benefit of this approach is that even a small amount of glycerol around 2 v% for a total amount of alcohol between 5 and 20 v% improves the Au NP size control towards lower sizes by favouring a faster formation. The strategy to use a smaller overall amount of chemicals to obtain small size NPs *ca.* 5 nm in diameter presents several potential benefits not only for scaling the NP synthesis but also for further uses in various applications.

The overall concept presented here could be extended to alternative alcohols and/or alcohols of different grades and materials. This strategy opens an opportunity for the rational development of more robust and yet simpler, more sustainable, safer and more tractable synthetic strategies for size-controlled NPs.

Data availability

The data supporting this article have been included as part of the ESI.†

Author contributions

Conceptualization: JQ; data curation: JCHL, ANP, LV, and JQ; formal analysis: JCHL, ANP, LV, and JQ; funding acquisition: JQ; investigation: JCHL, ANP, LV, and JQ; methodology: JQ; project administration: JQ; resources: JQ; supervision: AS and JQ; validation: JCHL, ANP, and LV; visualization: JCHL, ANP, LV, and JQ; writing – original draft: JQ; writing – review and editing: JCHL, ANP, LV, AS, and JQ.

Conflicts of interest

There are no conflicts of interest to declare.

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