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Preparation of nanomagnetic absorbent for partition coefficient measurement

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Abstract

In this paper, we report a new method based on supercritical carbon dioxide ($scCO_2$) to fill and distribute the porous magnetic nanoparticles with *n*-octanol in a homogeneous manner. The high solubility of *n*-octanol in $scCO_2$ and high diffusivity and permeability of the fluid allow efficient delivery of *n*-octanol into the porous magnetic nanoparticles. Thus, the *n*-octanol-loaded magnetic nanoparticles can be readily dispersed into aqueous buffer (pH 7.40) to form a homogenous suspension consisting of nano-sized *n*-octanol droplets. We refer this suspension as the *n*-octanol stock solution. The *n*-octanol stock solution is then mixed with bulk aqueous phase (pH 7.40) containing an organic compound prior to magnetic separation. The small-size of the particles and the efficient mixing enable a rapid establishment of the partition equilibrium of the organic compound between the solid supported *n*-octanol nano-droplets and the bulk aqueous phase. UV–vis spectrophotometry is then applied to determine the concentration of the organic compound in the aqueous phase both before and after partitioning (after magnetic separation). As a result, log *D* values of organic compounds of pharmaceutical interest determined by this modified method are found to be in excellent agreement with the literature data.

Keywords: Magnetic nanoparticle; Porous; Supercritical fluid; Partition coefficient; Magnetic separation

1. Introduction

A new nanotechnology based on magnetic separation using nanomagnet has recently been explored (Nam et al., 2003). This can be described as a two-step process, involving (i) tagging or labeling of desired biological/chemical entity on colloid magnetic nano-particle for recognition of complementary species in solution, and (ii) separation of the resulting solid entities via a fluid-based magnetic separation followed by regeneration of the species from the particle. The technique is now widely adopted in protein purification, immunoassays, pre-processing in polymerase chain reactions and pre-concentration of biological entities. Recently, applications of magnetic separation to catalysis and bio-catalysis areas in order to regenerate expensive catalyst species (Tsang et al., 2004) or enzymes (Gao et al., 2003) from reaction mixture have been particularly noted. On the other hand, there is very limited work of this new technique to seek applications to pharmaceutical industry.

For screening lead compounds in pharmaceutical industry, lipophilicity is a useful physicochemical parameter reflecting the transfer properties of a compound across biological membranes (Comer et al., 2001; Leo et al., 1969). This can be described by the *n*-octanol/water partition coefficient (log *D*), which is defined as the ratio of concentrations of a compound in all its forms between an aqueous phase (buffer) and an *n*-octanol phase. Shake-flask method is a simple and traditional method for the determination of the log *D* value (Comer et al., 2001; Leo et al., 1969). It is accepted as a standard procedure by the Organisation for Economic Cooperation and Development (OECD, 1992). The compound is introduced into the two immiscible phases, *n*-octanol and aqueous buffer solution, in a separator funnel. The funnel is then shaken until the partitioning equilibrium is achieved. After phase separation the concentration of the compound in each phase was determined. The drawbacks of this method include: a slow partitioning because of the bulk phases involved, labor intensive and emulsion formation upon shaking

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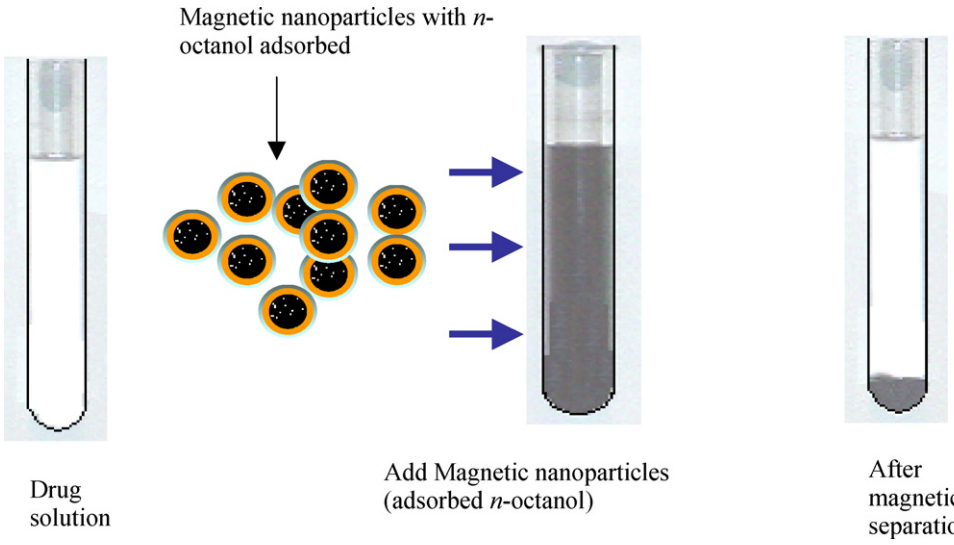


Fig. 1. A schematic diagram to illustrate the measurement of log *D* value of a drug compound using magnetic nanoparticles.

can interfere with the measurement. It has been deemed unsuitable for a high throughput screening (Danielsson and Zhang, 1996).

Thus, for the screening of log *D* of potential drug candidates we have recently developed a new miniaturization methodology for the determination of partition coefficient values of organic compounds in *n*-octanol/water by using porous magnetic nanoparticles (Tsang and Tam, 2005). The magnetic nanoparticles preloaded with a known amount of *n*-octanol (by gravimetric means) are dispersed in a bulk aqueous phase containing analyte compound. As a result, the small *n*-octanol droplets on the nanoparticles will create an excellent interface with the aqueous buffer phase, which significantly shortens the time required to achieve partition equilibrium. Then, the super-paramagnetic properties of the particles will allow a rapid magnetic induced precipitation and thus analysing the analyte concentrations before and after precipitation in the supernatant aqueous phase enables the log *D* to be worked out. Fig. 1 represents a schematic diagram of this magnetic nanoparticle approach.

However, with regard to the development of the method into a fully automatic procedure, volumetric delivery of known quantity of *n*-octanol-filled nanoparticles to drug solution would be more useful than our initially developed gravimetric procedure. This would involve creating a suspension containing known quantities of *n*-octanol by volumetric means for automation purpose. Here, we refer this preparation as the *n*-octanol stock solution. The quantity and distribution of *n*-octanol carried by the nanoparticles should be as homogeneous as possible in the stock solution. It is however, filling of the porous magnetic nanoparticles with pure *n*-octanol is rather difficult since the high viscosity of the *n*-octanol precludes the homogenous penetration and dispersion of the solvent into the pores, the surface coating of the particles will also lead to particle agglomeration. On the other hand, it is well-known that supercritical conditions for any compound can be achieved at a temperature and pressure above its critical values with existence of a single phase of high diffusion and penetration rates. Also, a chemically inert supercritical fluid such as carbon dioxide when used above its critical conditions can display solvency power common to conventional organic liquids as well as supporting exceptionally high transport properties common to gases (McHugh and Krukonis, 1986). The supercritical CO_2 is indeed demonstrated as a good solvent for hydrocarbons, aromatic hydrocarbons, halocarbons, aldehydes, esters, ketones, and alcohols of low molecular masses. It should be pointed out that the degree of solubility of chemical compounds in $scCO_2$ is critically affected by applied temperature and pressure. The solubility of *n*-octanol in $scCO_2$ was investigated by Nakaya et al. (2001). His work suggested that a large quantity of *n*-octanol can be completely dissolved into the $scCO_2$ fluid. In addition, it is also well-known that the $scCO_2$ shows a substantially higher diffusivity and lower viscosity than most organic solvents including liquid CO_2 (Kamat et al., 1993).

In this paper, we report the use of supercritical carbon dioxide as a solvent for filling internal pores of surface treated magnetic nanoparticles with *n*-octanol for the preparation of homogeneously dispersed *n*-octanol stock solution. Log *D* values of some typical drug compounds obtained by the *n*-octanol stock solution are demonstrated to be comparable with those of literature data.

2. Experimental

2.1. Chemicals used

The chemicals used for this work are listed as follows: *n*-octanol (99%), potassium diydrogenphosphate (+99%), 4-nitroanisole (97%), 4-nitrobenzyl alcohol (99%), 4-nitrophenol (98%), 3,5-dichlorophenol (97%) and chlorotrimethylsilane (CTMS) were obtained from Aldrich. Imipramine (98%), pyridine +99%), benzamide (99%), chlorpromazine (99%) and ibuprofen (98%) were obtained from Sigma. Quinoline (99%)

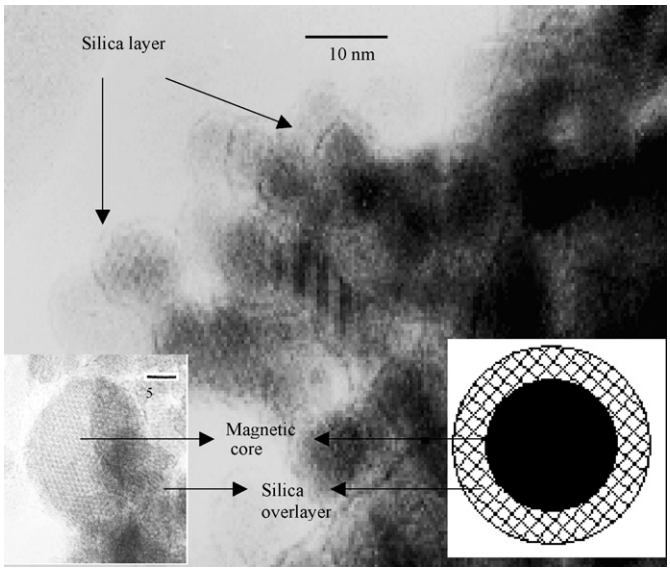


Fig. 2. TEM image and a model of the silica coated magnetic nanoparticles.

and aniline (99.8%) were obtained from Fisher-Acros. All of these chemicals were used without further purification. Porous silica encapsulated magnetic nanoparticles were synthesized in lab.

2.2. Preparation of porous nanomagnets

Porous silica encapsulated iron oxide magnetic nanoparticles as a nano-absorbent were synthesized using a microemulsion technique (Gao et al., 2003; Tsang and Tam, 2005). XRD reveals that the average diameter of the iron oxide nanoparticles is 9.9 nm, which is in an excellent agreement with a value of 9.1 nm as shown from the TEM image in Fig. 2. It can be seen that the iron oxide formed as an inner core (darker fringe) is covered by several loosely packed silicon–oxygen containing overlayers, which appears to be amorphous and porous (see Fig. 2, with TEM images and a scheme of the particle). Energy dispersive spectrometry (EDS) confirms that the elemental composition of these nanoparticles is $Fe_3O_{4.24}{\cdot}1.74SiO_2$. VSM analysis indicates that the saturation magnetization per kilogram of the magnetic iron oxide core of is of 76.16 emu/g. The value is close to the literature value of bulk Fe_3O_4, which is about 92 emu/g. Also, the measured remanence of these nanoparticles is 4.5 A m^2/kg and coercivity is 15.3 kA/m. Magnetic remanence represents a magnetic field remained in material after it is exposed to an external magnetic field. The coercivity is defined as the magnetic property of a material which resists its de-magnetization. High magnetic remanence and high coercivity values indicate that the material is a 'hard' type of magnetic material. Thus, our nanoparticles are characterized with low remanence and coercivity values suggest that the particles will only behave as little magnets upon exposure to a strong external magnetic field leading to precipitation. Without the magnetic field the 'soft' nanoparticles will return as colloidal dispersed particles. Thus these data clearly agree that the nanoparticles exhibit superparamagnetic behavior at room temperature as expected from the nano-sized iron oxide particles. It is important to note that the super-paramagnetism means that there is no remaining induced magnetism due to repeated exposure of magnetic field.

2.3. Filling octanol via supercritical fluid

In this $scCO_2$ approach, the delivery of *n*-octanol to the porous nanoparticles was carried out using the set-up shown in Fig. 3. The experiment was carried out in a stainless steel Parr auto-clave. In a typical experiment, about 60.7 mg dried porous silica encapsulated nanoparticles were placed in a sample holder. A known amount of *n*-octanol (from 30 μl to 2 ml pre-saturated with buffer solution) was then added into the holder using a micro-pipette. The autoclave vessel and the sample holder were alternatively charged and flushed with $scCO_2$ by opening and closing the valves between the two compartments and external outlets before the vessels were brought up to a desirable pressure (15 MPa). The temperatures of the autoclave and sample holder were both maintained at 313 K. After 2 h, the high pressure of the system was released to the atmosphere very slowly. It is believed that the *n*-octanol will gradually return to liquid state in the pores of the nanoparticles at ambient pressure. A gravimetric method was employed to work out the precise *n*-octanol loading on the solid. It is noted that temperature and pressure were chosen for the primarily reason of *n*-octanol solubility in the supercritical carbon dioxide phase. It is known that the solvency of a supercritical phase is mainly governed by temperature and pressure. The higher the pressure used the more liquid-like the phase would be (akin to conventional organic liquids) if the temperature is above the critical point. In the case of carbon diox-

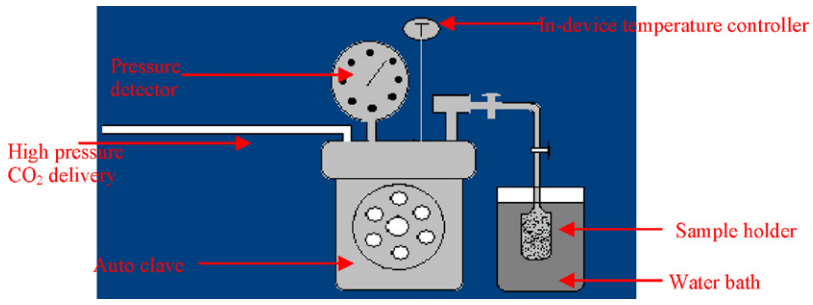


Fig. 3. Experimental set-up for the deposition of *n*-octanol onto porous nanoparticles via supercritical CO_2 delivery.

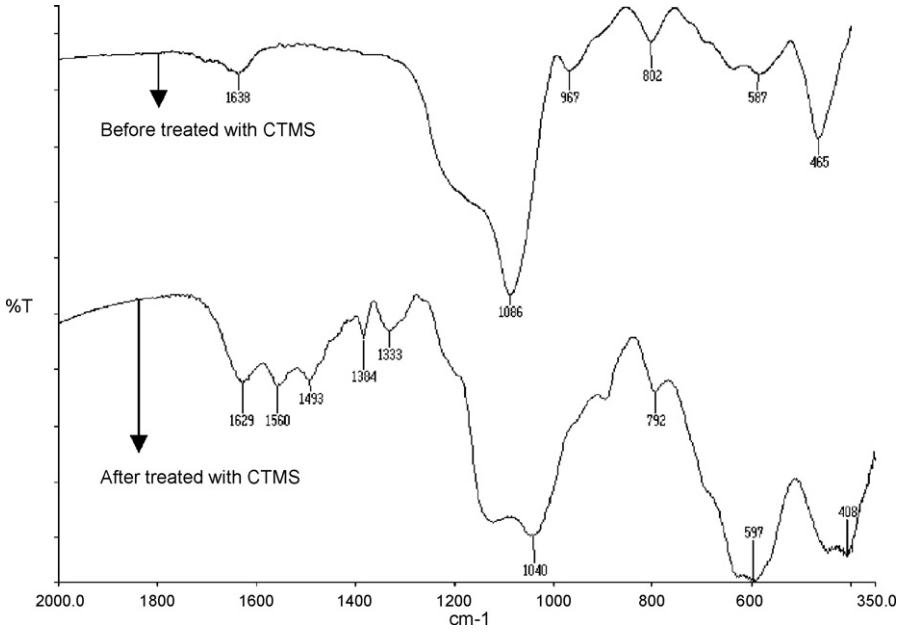


Fig. 4. FTIR spectra of the silica encapsulated nanoparticles before and after CTMS treatment.

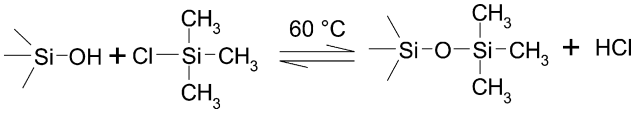
ide 304.3 K and 7.38 MPa are the theoretical critical temperature and pressure. Without carrying out optimisation for a *n*-octanol solubility study, 313 K and 15 MPa were employed since this applied pressure is the highest allowed to be used above the critical temperature in our apparatus. These conditions will ensure all the *n*-octanol used in our experiments would be totally dissolved in the supercritical carbon dioxide phase as the amount of *n*-octanol used falls below the maximum solubility value of *n*-octanol in $scCO_2$ defined in the paper of Nakaya et al. (2001).

In a typical experiment, 71.2 mg dried but surface treated nanoparticles were placed in the sample holder. 22.3 μl *n*-octanol was allowed to mix with the particles. After loading the *n*-octanol to the nanoparticles, the solid was placed in a volumetric flask. Aqueous buffer solution (pre-saturated with *n*-octanol, pH 7.40) was added to prepare a homogeneous 5.00 ml magnetic nanoparticle stock solution.

Partition coefficient measurements of selected drug compounds were carried out as follows: in each experiment, 1.00 ml extract from the stock solution was delivered by an adjustable-volume digital pipttee into a polyethylene vial and a further 3.00 ml drug solution was then mixed with the extract. This vial was sealed and agitated in an electronic orbital shaker for about 20 min. After establishing partition equilibrium induced precipitation of the nanoparticles from the resulting solution was achieved using a permanent magnet ($BH_{max} = 38$ MG Oe) located at the bottom of the vial. UV–vis absorption spectra of the compound at aqueous phase before and after partition were collected from the supernatant solution without carrying out any physical separation.

2.4. Surface treatments

About 10 nm porous silica encapsulated iron oxide magnetic nanoparticles as a nano-absorbent were synthesized using a microemulsion technique (Gao et al., 2003). In order to fill and distribute the internal pores of magnetic particles with the relatively hydrophobic *n*-octanol, the surface functionalities of the particles could not be too hydrophilic. Thus, capping surface hydroxyl groups on the silica surface was carried out using chlorotrimethylsilane (CTMS) as follows:



In a typical experiment, 2.00 g silica encapsulated nanoparticles were washed, dried and put in a 50 ml narrow-neck glass flask. An excess amount of CTMS (4.00 ml) was added into the flask as well. The flask was sealed with an aluminium foil and placed in an oven at 60 °C overnight. After cooling down to room temperature, the CTMS treated nanoparticles were then washed with water, acetone and dried under a nitrogen atmosphere. FTIR spectrometry was used to monitor the degree of capping of the surface hydroxyl groups. Fig. 4 shows the corresponding FTIR spectra of the nanoparticles before and after the CTMS treatment. As shown in Fig. 4, it can be clearly seen that the absorption band at 967 cm^{-1} due the asymmetric stretching of hydroxyl groups on silica surface (Si–OH) disappeared upon the CTMS treatment. In addition, there was a new absorption band at about 850 cm^{-1} which can be assigned to the stretching mode of methyl groups (Bu and Rhee, 2000; Kamitsos et al., 1993). Thus, this provides a strong proof that the hydroxyl groups on the silica coating surface were successfully capped by the CTMS molecules.

2.5. Thermal gravimetry

Thermal gravimetry analysis (TGA) was used to measure the amount of the adsorbed *n*-octanol taken up by the nanoparticles. Thus, the *n*-octanol-filled nanoparticles were heated from room temperature to 1073 K (800 °C) under nitrogen. The weight lost

Table 1
A typical added ingredients via $scCO_2$ approach

Nanoparticle (mg)	*n*-Octanol content (mg)	Volume of *n*-octanol (μl)	Drug solution (ml)	Phase ratio (water:*n*-octanol)
71.2	18.4	22.3	2.23	100:1

of the filled nanoparticles was attributed to the disappearance of *n*-octanol (controlled experiments showed almost no weight lost due to any moisture in the sample if it was properly dried).

3. Results and discussion

Fig. 5 shows the TGA analysis curve, which clearly suggests 0.54 ml *n*-octanol could be taken up by 1.0 g of the magnetic nanoparticles. To carry out the partition coefficient determination experiment, the *n*-octanol-filled nanoparticles were first dispersed in a known amount of buffer to form a homogenous *n*-octanol stock solution in a polyethylene vial. Then, an appropriate amount of drug solution was added into the vial to maintain the water/*n*-octanol phase ratio at 100. (It is noted that this is a typical ratio used in the traditional shake-flask method as most drug compounds are rather lipophilic in properties. This, in this new method, the same ratio is used.) Table 1 shows a typical quantity of ingredients for the determination of log *D*.

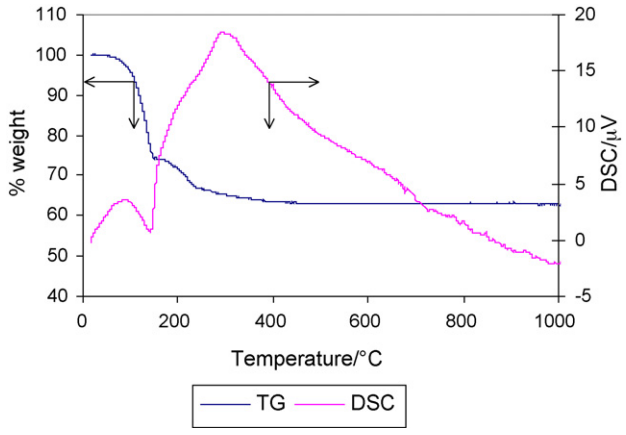


Fig. 5. TGA analysis of nanoparticles-filled with *n*-octanol. 36.98% weight lost was recorded after heating up the particles to above 673 K.

It was noted that the nanoparticles were homogeneously dispersed into the bulk aqueous phase throughout these mixing processes. The vial was slightly shaken in an electronic orbital shaker for about 20 min. Magnetic induced precipitation of the nanoparticles was then achieved by using a permanent magnet (BH_{max} = 38 MG Oe) located at the bottom of the vial. UV–vis absorption spectra of the compound at the aqueous phase before and after partition were collected. All absorption data were background corrected before used. The log *D* value was calculated using Eq. (1) (the same equation was used for the shake-flask method).

$$\log D = \log \left[\frac{V_w}{V_o} \times \frac{A_1 - A_2}{A_2} \right] \quad (1)$$

where A_1 and A_2 represent the UV–vis absorption values of the compound in the aqueous phase before and after partition, respectively while V_w/V_o represents the water:*n*-octanol phase ratio.

Table 2 and Fig. 6 clearly show an excellent correlation of the results from the present method compared to the literature data. The good agreement in the log *D* value of strong hydrogen

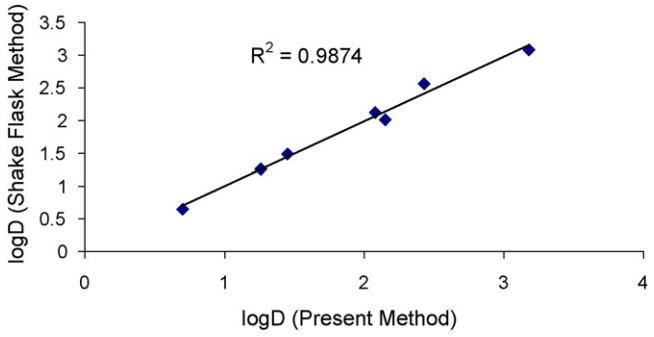


Fig. 6. A correlation curve of log *D* values measured by the present method compared with the log *D* values measured by shake-flask method at reading.

Table 2
Comparison of *n*-octanol–water partition coefficients (log *D*) obtained by the present method with the shake-flask method and corresponding literature values at pH 7.40 (confidence level is 95%)

Drug	Present method	Shake-flask method		Literature data (Gao et al., 2005)
		Reading	AstraZeneca[a]	
Benzamide	0.70 ± 0.41	0.65 ± 0.01	0.66	0.64
Chlorpromazine	3.18 ± 0.45	3.08 ± 0.01	3.20	NA[b]
Imipramine	2.43 ± 0.49	2.56 ± 0.04	2.50	2.40
4-Nitroanisole	2.15 ± 0.07	2.01 ± 0.01	NA[b]	2.03
4-Nitrobenzylalcohol	1.26 ± 0.11	1.26 ± 0.01	NA[b]	1.26
4-Nitrophenol	1.45 ± 0.03	1.49 ± 0.01	1.48	1.38
Quinoline	2.08 ± 0.07	2.12 ± 0.01	2.02	2.03

[a] Log *D* values were determined by shake-flask method in AstraZeneca.
[b] Not available.

bond acceptor such as quinoline (see Table 2) strongly suggests that majority of the surface –OH groups on the internal surface of the magnetic nanoparticles has been removed (capped) by the surface treatments. It is believed that the *n*-octanol stock solution procedure described here could offer a robotic friendly miniaturization platform, which would be well adopted as an automation method for a high throughput log *D* screen of drug candidates.

4. Conclusion

A novel methodology in filling porous silica encapsulated iron oxide nanoparticles with *n*-octanol via supercritical fluid to determine the partition coefficient of organic compounds in aqueous phase/*n*-octanol has now been developed. It is noted that the key challenges for the success application of magnetic separation technology for this measurement including preparation of porous magnetic iron oxide nanoparticles (iron oxide particle with the size larger than 100 nm will not show the super-paramagnetic properties which is absolutely essential for reversible magnetic separation) and filling them with *n*-octanol (preparation of homogeneously dispersed stock solution via supercritical fluid) are successfully synthesized. Our experimental results clearly demonstrate the usefulness of this new modified method for the actual determination of partition coefficient values, which requires only a very small quantity of compound, solvent and the nanoparticles.

Acknowledgements

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