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Magnetic Janus Particles Synthesized by Droplet Micro-magnetofluidic Techniques for Protein Detection

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Magnetic droplets on a microfluidic platform can act as micro-robots, providing wireless, remote, and programmable control. This field of droplet micro-magnetofluidics (DMMF) is useful for droplet merging, mixing and synthesis of Janus structures. Specifically, magnetic Janus particles (MJP) are useful for protein and DNA detection as well as magnetically controlled bioprinting. However, synthesis of MJP with control of the functional phases is a challenge. Hence, we developed a high flow rate, surfactant-free, wash-less method to synthesize MJP by integration of DMMF with hybrid magnetic fields. The effect of flow rate, flow rate ratio, and the hybrid magnetic field on the magnetic component of the Janus droplets and the MJP was investigated. It was found that the magnetization, particle size, and phase distribution inside MJP could be readily tuned by the flow rates and the magnetic field. The magnetic component in the MJP could be concentrated after mixing at flow rate ratio values less than 7.5 and flow rates less than 3 ml/h. The experimental results and our simulations are in good agreement. The synthesized magnetic-fluorescent Janus particles were used for protein detection, with BSA as a model protein.

Introduction

Droplet microfluidics^{1–4} (DMF) is an emerging technique for the manipulation of discrete quantities of matter as well as fluids⁵ on a microfluidic platform. Each droplet acts as a microscale isolated reaction container, with multiplexing capabilities in a large volume range (pL to μ L)^{3, 6}. Hence, DMF is a very promising technique for a range of applications. DMF has been utilized for enzyme inhibition assays⁷, biochemical analysis⁸, and microbiological applications⁹. DMF based single cell analysis has been utilized for detection of protein expression¹⁰, cell culture studies, cell lysis, drug efficacy studies¹¹, single cell transcriptomics¹², stem cell engineering¹³ and single-cell barcoding¹⁴. DMF techniques which are employed for cancer research include deoxyribonucleic acid (DNA) studies, protein marker detection¹⁵ as well as single molecule analysis¹⁶. DMF is also recognized as a novel platform for the synthesis of hydrogel microparticles¹⁷, photocrosslinkable materials¹⁸, polymer particles¹⁹, and multifunctional particles²⁰. It can also be used to synthesize particles for drug delivery applications^{21, 22}, with control of particle morphology²³ and mechanical properties²³.

Specifically, there is increasing interest in deploying DMF techniques for the synthesis of Janus particles^{24–26}. Janus particles are a class of multifunctional particles, where the constituent phases are accessible and available for use. Janus particles overcome a key limitation of core-shell structures,

wherein one phase (core) is surrounded by another phase (shell), decreasing the net advantage. In contrast, Janus particles provide anisotropic control of the constituent phases with direct access to the phases. This can result in non-trivial, antagonistic, and attractive combination of properties, e.g., hydrophilic-hydrophobic, magnetic-plasmonic, ferromagnetic-diamagnetic, magnetic-optical, and magnetic-photonic.

However, controlled synthesis of Janus particles is challenging. Earlier work on the synthesis of Janus particles include the work of Heida et al.²⁷ on the mechanical properties, functionalization and cross-linking density of these particles. Zhao et al.²⁸ reviewed photonic crystal based Janus particles. Nisisako²⁴ reviewed microfluidic approaches to fabricate Janus droplets and Janus particles. Lone et al.²⁵ summarized DMF approaches for Janus particle fabrication. Kim et al.²⁹ reviewed DMF based synthesis methods for functional microparticles.

Synthesis begins with active or passive droplet generation^{1–4}, followed by integration of the various components. Various methods utilized for the synthesis of Janus particles are described in **Table 1**.

Um et al.³⁰ used an electric charge concentration (ECC) technique for droplet generation, control, and fabrication of Janus particles of size of ~ 1 mm. Their method operated in three modes (i.e., attaching, uniform, and bursting) by tuning flow rate, voltage, and oil surface-nozzle distance. They combined their technique with ultraviolet (UV) polymerization to fabricate polyethylene glycol diacrylate (PEGDA) based Janus particles containing CaCl_2 : alginate. Lan et al.³¹ used a flow focusing configuration to fabricate alginate based Janus particles of magnetite: CdSe/ZnS quantum dots. Fabrication was performed by ionic crosslinking of the alginate phase by the Ca^{2+} containing continuous phase. These Janus particles were then

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utilized for DNA assays. Hessberger et al.³² utilized a capillary-based microfluidic setup integrated with UV polymerization to fabricate Janus particles capable of actuation. The Janus system

was composed of a hydrophobic liquid crystalline part and a hydrophilic polyacrylamide network, with a particle size of ~ 700 μm .

Table 1: Comparison of various methods utilized for the synthesis of Janus particles. Notations: D_p : particle size, PPMZ: photopolymerization, DNA: deoxyribonucleic acid, PEGDA: poly(ethylene glycol diacrylate), HSA: human serum albumin, PTPGDA: poly(tripropylene glycol diacrylate), μPMMA : PMMA microparticles, nano-TiO₂: TiO₂ nanoparticles, or nano-Fe₃O₄: magnetite nanoparticles, PNIPAAm: poly(N-isopropylacrylamide).

Research Group	Approach/ Method	D_p (μm)	Janus Components	Applications/ Advantage
S.J. Lee ³⁰ Z.K. He ³¹	PPMZ+ electric charge concentration Ionic cross-linking of the alginate by Ca^{2+} solution	1000 60	CaCl_2 : alginate in PEGDA magnetite: CdSe/ZnS quantum dots	electrostatic self-assembly DNA assays
R. Zentel ³²	PPMZ+ capillary microfluidic	700	hydrophobic liquid crystals: hydrophilic polyacrylamide	liquid crystals
T.M. Swager ³³	PPMZ+ complex emulsions tuned by interfacial tensions	100	four-phase configuration with nano-Fe ₃ O ₄	drug release at tumours, sensors camouflage, lenses
T. Takeuchi ³⁴ Z.Q. Chang ³⁵ M. Ardekani ³⁶	Inverse suspension PPMZ Drying the droplets at 90°C for 24 h Off-chip ionic cross-linking by hot glycerol + barium acetate	≥ 1000 160 140	molecularly imprinted microgels carbon black: PTFE alginate: PNIPAAm	human serum albumin detection optical and electrical control shape controlled particle fabrication
S.Y. Park ³⁷ S. Bon ³⁸	PPMZ on DMF platform Single emulsion DMF + separation under magnetic field and gravity	100 500	Smooth: porous PTPGDA μPMMA : nano-TiO ₂ , or nano-Fe ₃ O ₄	self-assembly of particles temperature and pH based entrapment and colloid release
S. Chen ³⁹ J.W. Smith ⁴⁰ P.S. Doyle ⁴¹ P.S. Doyle ⁴²	PPMZ + control of interfacial forces Fluidic nano-precipitation Continuous flow lithography Stop-flow lithography	150 0.5 130 250	photonic, polystyrene: nano-Fe ₃ O ₄ hydrophilic: hydrophobic PEGDA+ rhodamine PEGDA porosity controlled PEGDA	magnetic multicolour patterns drug delivery shape controlled particles transferrin detection

Figure 1: (a) Schematic of DMMF based Janus particle synthesis. Magnetic Janus droplet (MJD) generation was performed by two configurations of flow focusing geometry: (i) LoC configuration consisting of a PMMA microfluidic chip, with dimensions: $a=100$ μm , $W=300$ μm . (ii) Capillary DMF configuration consisting of a micro flow focusing device with dimensions: $a=150$ μm , $W=250$ μm . The continuous phase (CP) consists of hexane. The dispersed phase (DP) consists of co-flowing streams of magnetic polymeric phase (MP) and a polymeric phase (PP). (b) Magnetic field distribution along the xz direction. The hybrid magnetic field ' $H=H_o+H_{no}$ ' was generated by two Nd-Fe-B magnets facing each other. Janus particle synthesis was performed at 440 mT by photopolymerization under a UV LED. MJD flow is along the $+x$ direction. The hybrid magnetic fields and UV LED are along the y and z directions, respectively. The position $x=0$ mm was assigned to the magnetic field centre, denoted by the dotted red line.

Zarzar et al.³³ employed dynamic control of complex emulsions for Janus particle synthesis by tuning the interfacial tensions of the constituent phases. They photopolymerized a four-phase emulsion, resulting in magnetite-based Janus particles. Applications include controlled drug release at tumours, camouflage, tunable lenses, and sensors. Takimoto et al.³⁴ used inverse suspension polymerization to fabricate molecularly imprinted submillimeter microgels. They used water-in-oil (W/O) droplets containing a water-soluble monomer in a microchannel and photopolymerized the droplets using UV light. These microgels were utilized for

detection of bovine serum albumin (BSA) and human serum albumin (HSA).

Li et al.³⁵ fabricated Janus microspheres of carbon black: polytetrafluoroethylene (PTFE) by drying the droplets in an oven at 90°C for 24 h using a simple capillary microfluidic device. The optical and electrical anisotropy of the particles was controlled by tuning flow rates and flow rate ratios. Detailed investigation of the electro-responsive properties was also performed. Hu et al.³⁶ utilized off-chip ionic cross-linking to fabricate shape controllable alginate: poly(N-isopropylacrylamide) (PNIPAAm) Janus microgels. Droplet

generation was performed by a microfluidic co-flowing configuration. Ionic cross-linking was performed by a hot aqueous solution of glycerol + barium acetate. The shape and surface morphology of the particles was tuned by changing initial droplet size and glycerol concentration in the crosslinking solution. Kim et al.³⁷ used the DMF approach, combined with directional UV curing, to synthesize asymmetric porous Janus particles. Their Janus structures were prepared with polytripropylene glycol diacrylate (PTPGDA) as the matrix, exhibiting a smooth structure in one part and porous structure in the other part. They used a flow focusing geometry for droplet generation and polymerized the particles using directional UV light, resulting in Janus particles of size ~ 100 µm. Chen et al.³⁸ utilized a single emulsion DMF approach to fabricate Janus particles. Their Janus system was composed of branched polymers containing colloidal particles (PMMA microspheres, TiO₂ particles, or magnetite nanoparticles, **Table 1**). The Janus configuration was achieved by separation under magnetic field and gravity. They used a co-flowing geometry for droplet generation, combined with the evaporation of water, resulting in Janus particles of ~500 µm size.

Yu et al.³⁹ synthesized photonic Janus particles by photopolymerization of EOTMPTA based polystyrene: magnetite nanoparticles. They used a balance between interfacial forces to control the geometry between biphasic droplets. Swelling and the photonic colours of the particles was controlled by acrylic acid. These particles could be used to create multicolour patterns controlled by a magnetic field. Xie et al.⁴⁰ synthesized Janus particles containing a hydrophilic: hydrophobic drug, useful for drug delivery applications. They developed a fluidic nano-precipitation system for one-step fabrication of poly (lactic-co-glycolic acid) (PLGA) based sub-micrometer Janus particles and used a T-junction configuration with two parallel inlets for the dispersed phases, resulting in a Janus particle size of ~400 nm.

The Doyle group used continuous flow lithography⁴¹ and stop flow lithography⁴² to fabricate barcoded Janus particles. For the continuous lithography approach, they used a single phase based synthesis of morphologically complex multifunctional particle synthesis. They investigated particle size control by changing the flow rates and particle shape control by lithography masks. For the stop flow lithography approach, they used PEGDA as the hydrogel medium which contains the constituent Janus components. The synthesized Janus barcodes were used for protein detection.

Though various approaches have been utilized in the past, magnetic fields have not yet been used for dynamic control of Janus droplets to synthesize Janus particles. Our droplet micro-magnetofluidic (DMMF) technique offers a versatile approach to synthesize Janus particles with wireless, programmable and remote control of droplets. We developed a DMMF technique combined with hybrid magnetic fields to synthesize magnetic Janus particles (MJP). The hybrid magnetic field ($H = H_o + H_{no}$) is a combination of uniform magnetic field (H_o) and non-uniform magnetic field (H_{no}). Our method is useful to perform controlled polymerization on LoC and capillary microfluidic platforms. The capillary microfluidic platform was found to be adaptable,

flexible and low cost. Hence, we used a capillary DMMF platform in the current studies. The effect of flow rate and applied magnetic field on mixing, concentration of the magnetic phase within the droplets, as well as the magnetization, particle size and distribution of the magnetic component in the MJP were investigated. The properties of the MJP were studied by VSM, FTIR, DSC, TGA and SEM techniques and protein detection demonstrated with bovine serum albumin as a model protein.

Experimental Methodology

The experimental setup, materials used, the microfluidic chip, and experimental parameters are summarized in this section.

Materials

We used immiscible fluids to generate magnetic Janus droplets (MJDs) (**Figure 1**). Hexane was used as the continuous phase (CP). The dispersed phase (DP) consists of (i) a magnetic phase (MP) of EMG 507 ferrofluid (Ferrotec, Singapore) for magnetic control and (ii) a polymeric phase (PP) for protein detection (**Table 2**). Two photopolymerizable solutions (PPS) of polyethylene glycol diacrylate (PEGDA, MW 575 and MW 700) containing 2-hydroxy-2-methylpropiophenone (HMP) as the photoinitiator were prepared, viz., (i) PPS5= PEGDA 575 + 13 % v/v HMP and (ii) PPS7= PEGDA 700 + 13 % v/v HMP.

Table 2: Compositions and viscosities of liquid phases. Brookfield rheometer (model: LV-DV3T, spindle: CPA 40z, sample volume of 0.5 ml) was used to measure viscosity. Density measurements were performed by the rapid micro-syringe method⁴³. Notation: (i) PPS5= polyethylene glycol diacrylate (MW 575) + 13 % v/v HMP and (ii) PPS7= polyethylene glycol diacrylate (MW 700) + 13 % v/v HMP. Where, HMP= 2-hydroxy-2-methylpropiophenone photoinitiator, PPS: photopolymerizable solution, and MW: molecular weight.

Sr.	Liquid Phase	Composition	Viscosity (mPa.s)	Density ($\times 10^3 \text{ kg/m}^3$)
1	Ferrofluid	EMG 507 ferrofluid	1.44 ± 0.01	1.118 ± 0.004
2	Continuous Phase (CP)	n-Hexane	0.31 ± 0.01	0.667 ± 0.007
3	Polymeric Phase (PP)	[5ml PPS5+ 2.5ml AA + 2.5ml EG] + 0.5mg/ml rhodamine 6G	35.83 ± 0.36	1.102 ± 0.005
4	Magnetic Phase (MP)	2.5ml PPS5 + 4.5ml PPS7 + 2ml EMG 507 + 2.5ml DI water + 1ml 1% (v/v) Tween 20 (in DI water)	22.25 ± 0.23	1.133 ± 0.002
5	Dispersed Phase (DP)	0.25 ml Polymeric phase + 0.25 ml Magnetic phase	31.20 ± 0.31	1.119 ± 0.006

Ethyl(dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were utilized for protein immobilization experiments. Bovine serum albumin (BSA) and fluorescein isothiocyanate (FITC) tagged BSA (FITC-BSA) were used as model proteins. All chemicals were purchased from Sigma-Aldrich, Singapore unless otherwise specified. The magnetic phase consists (**Table 2**) of 2.5 ml PPS5 + 4.5 ml PPS7 + 2 ml EMG 507 + 2.5 ml DI water + 1 ml 1% (v/v) Tween 20 (in DI water). The composition of the polymeric phase was optimized for enhanced protein binding, the details are described in the results section.

EDC/NHS protocol for protein immobilization

The EDC/NHS protocol was used for covalent immobilization of protein (BSA and FITC-BSA as model proteins) on the Janus particle or hydrogel surfaces. The coupling protocol was performed by adding 2mM EDC and 5mM NHS to the magnetic Janus particle in PBS 1X buffer for 3 h. After 3 h, the particles were washed thrice in PBS 1X buffer. Protein solution (in PBS 1X) was added immediately and kept at 4°C for 3 h. The particles were then thoroughly washed thrice with PBS 1X to remove weakly bonded or physically entrapped protein. A fluorescent microscope (Olympus IX73) integrated with a TOUPCAM camera (ToupTek photonics, Model: UCMOS03100KP, P/N:TP603100A) was used to capture fluorescent images of the particles in FITC mode. ImageJ was used to quantify the measurements.

Viscosity measurements of the liquid phases were performed by a Brookfield rheometer (model: LV-DV3T, sample volume: 0.5 ml, spindle: CPA 40z) (Table 2). The measured density⁴³ of the ferrofluid was $1.118 \pm 0.004 \times 10^3 \text{ kg/m}^3$. The properties of the water-based ferrofluid EMG 507 are summarized in Table 3.

Table 3: Properties of EMG 507 ferrofluid. Note: *Properties as provided from supplier data sheet (Ferrotec Singapore), #Measured properties

Quantity	Values
#Viscosity (mPa-s)	1.44±0.01
#Density ($\times 10^3 \text{ kg/m}^3$)	1.118±0.004
*Saturation Magnetization (mT)	11
*Initial Magnetic Susceptibility (SI units)	1.63
*Magnetic Particle Concentration (% volume)	2
*Particle Diameter (nm)	10

Experimental Parameters

The effect of flow rates, magnetic field strength, and synthesis parameters to produce the Janus particles was studied at different flow rate ratios (Ψ) (Table 4).

Table 4: Parameters and notation. (Refer Table 2 for compositions and notations). MJD: magnetic Janus droplets, MJP: magnetic Janus particles. Q_{cp} , Q_{MP} , and Q_{PP} are flow rates of the continuous phase, magnetic phase, and polymeric phase, respectively. The flow rate ratio (Ψ) was defined by $\Psi = Q_{cp}/(Q_{MP} + Q_{PP})$ with $Q_{dp} = Q_{MP} + Q_{PP}$. Hexane was used as the continuous phase (CP). Dispersed phase consists of magnetic phase (MP) and polymeric phase (PP)

Sr	Flow rate ratio (Ψ)	Q_{cp} ($\mu\text{l/h}$)	Q_{MP} ($\mu\text{l/h}$)	Q_{PP} ($\mu\text{l/h}$)	MJD Set	MJP Set
1	2.5	1000	200	200	MJD1000	MJP1000
2	5	2000	200	200	MJD2000	MJP2000
3	6.25	2500	200	200	MJD2500	MJP2500
4	7.5	3000	200	200	MJD3000	MJP3000
5	10	4000	200	200	MJD4000	MJP4000
6	6.25	5000	400	400	MJD5000	MJP5000

The flow rate ratio (Ψ) was defined as the ratio of continuous phase flow rate (Q_{cp}) to the dispersed phase flow rate (Q_{dp}). The flow rate Q_{cp} is the total flow rate of the continuous phase (Figure 1). The flow rate Q_{dp} is the combined flow rate of the magnetic phase (Q_{MP}) and the polymeric phase (Q_{PP}). Competition between the magnetic volume force and the

hydrodynamic force on the magnetic particles and Janus droplets was investigated through the effect of different flow rate ratios at H_0 of 440mT. The parameters used for investigation and synthesis are summarized in Table 4.

Experimental Setup

A droplet micro-magnetofluidic setup (Figure 2) was developed for the LoC synthesis of magnetic Janus particle; synthesis could be magnetically controlled by hybrid magnetic fields ($H_0 + H_{no}$). The main components of this system are (i) a Janus droplet generation unit, (ii) a magnetic control unit, (iii) a polymerization unit, (iv) a high-precision control stage, and (v) a collection unit. These components are described in the following subsections.

Figure 2: Experimental setup of droplet micro-magnetofluidic based magnetic Janus particle synthesis, consist of (a) Janus droplet generation unit, (b) magnetic control unit, (c) polymerization unit, (d) high-precision control stage, and (e) collection unit. For droplet generation, two designs were used, (i) PMMA microfluidic chip based LoC flow focusing design ($100\mu\text{m} \times 100\mu\text{m}$ cross-section), (ii) capillary microfluidic design ($150\mu\text{m}$ thru hole diameter).

Magnetic Janus droplet generation unit and analysis

Two platforms were developed for magnetic Janus droplet generation, viz., (i) LoC based flow focusing design and (ii) capillary microfluidic-based micro flow focusing. Both designs have their own advantages.

LoC Flow Focusing Design. A poly(methyl methacrylate) (PMMA) based LoC flow focusing system was fabricated by a micro-

milling technique and bonded by thermal bonding⁴⁴ at 95°C under a load of 50 kg for a duration of 16 min. The dimensions and schematic of the setup are described in **Figure 1**. The LoC platform offers good droplet size control (**Figure 3**); however, it is limited by the narrow range of flow rates (**Figure 2**) and low droplet generation rate, since the flow rates are less than 100 $\mu\text{l/h}$. The LoC platform is also difficult to clean and reuse on blockage, which can occur due to stray polymerization, the LoC has to be replaced in the event of a blockage. Hence, we found that the LoC platform was less suitable for our experiments.

Figure 3: LoC Magnetic Janus droplet (MJD) generation (a) MJD micrograph (scale bar= 500 μm) and (b) droplet size v/s flow rate ratio. Where, CP= continuous phase, DP= dispersed phase, Q_{cp} =continuous phase flow rate, Q_{dp} =dispersed phase flow rate. Q_{cp} and Q_{dp} are in $\mu\text{l/h}$.

Capillary-based Droplet Microfluidic Platform. The capillary droplet microfluidic platform consists of a micro flow focusing connector with 150 μm thru-hole (P-891-microcross PEEK, IDEX, Singapore) connected by fluorinated ethylene propylene (FEP) microcapillaries, which are chemically inert to many solvents. FEP microcapillaries were used as inlet (500 μm diameter, P/N: 1548, IDEX, Singapore) and outlet microchannels (250 μm diameter, P/N: 1527, IDEX, Singapore). The capillary-based design was found to be affordable, simple, and flexible. It offers a large range of flow rates, resulting in a broad range of droplet and particle sizes, and is compatible with all the chemicals used for Janus particle synthesis. Flow rates of up to 10 ml/h can be utilized for droplet generation and particle fabrication by this platform. This device found to be very handy for cleaning blockages and can be recovered by purging at high flow rates or changing the FEP microcapillary.

Syringe Pump. KDS (model: Gemini 88 dual rate) and New Era (model: NE-1010 and NE-1002x, Achema, Singapore) syringe pumps were used to control flow rates. Exmire Luer lock gastight syringes (volume: 2.5 ml, P/N: MS*GLL250) were used to feed the flow to the microchannels. FEP microcapillaries were used for connections.

High-Speed Imaging and analysis. A Phantom Miro Camera (Model: M320s) integrated with Olympus IX73 microscope was used for high-speed imaging. High-speed imaging was recorded at a frame rate of 1000 fps and a resolution of 1920×600 pixels. Image acquisition and analysis of recorded videos was performed by ImageJ and Phantom camera control (PCC) software. Points in all graphs denote the mean of the measurements, with the error bar denoting the standard error of the mean⁴⁵.

Magnetic Control Unit

We used two Nd-Fe-B permanent magnets with dimensions 25mm×12.5mm×12.5mm (length× width× height) to generate hybrid magnetic fields. The strength and distribution of the hybrid magnetic fields can be controlled by adjusting the distance between the two magnets. The magnetic field distribution was measured along the $\pm x$ direction by a LakeShore Gaussmeter (model: 410). The distribution of experimental and simulated hybrid magnetic fields is shown in **Figure 1b**, with a H_0 field of 440 mT in the region $-8 \text{ mm} \leq x \leq 8 \text{ mm}$.

LoC Polymerization Unit

The polymerization unit was utilized for LoC polymerization of the Janus droplets. The unit was developed by integrating a UV LED with a 20X objective. A high-intensity UV-LED of 4 mm beam size was utilized as the UV light source (Agiltron UV LED, Model: SUVA-011111021) (**Table 5**).

Table 5: Specifications of UV LED Source (Supplier datasheet: Agiltron UV LED, Model: SUVA-011111021)

Sr	Parameter	Value
1	Wavelength (nm)	365±5, 385±5
2	UV irradiance (W/cm^2)	4.0 – 5.0
3	Cure time Max (s)	60
4	UV spot size (mm)	4
5	UV cure distance (mm)	20
6	Cooling method	air blow

High-Precision Stage

Control of the location of polymerization is a challenge, which we addressed by using a high precision control stage for control along the x, y directions. The UV light was incident along the z-direction and focused using the z-adjustment of the microscope. Hence, this setup offers precise, 3D control of polymerization.

Collection Unit.

The fabricated particles were collected at the exit in antistatic weighing dishes/pouring boats. The magnetically controlled LoC polymerization unit utilized magnetic Janus droplets to fabricate the desired magnetic Janus particle. This system could synthesize Janus particles in the size range of 50 μm to 1000 μm , with flow rates up to 10 ml/h (without ferrofluid) and 5 ml/h (with ferrofluid).

Theory and Simulation

Rosensweig's⁴⁶ continuum hypothesis was used to model the behaviour of Janus droplets in hybrid magnetic fields. A droplet micro-magnetofluidic 2D numerical model was developed with droplet motion in the positive x direction and the hybrid magnetic field in the y-direction (**Figure 1**). The following subsections describe the theoretical background.

Equation of motion

Rosensweig's continuum model contains an additional force term of the magnetic volume force ($\mathbf{F}_m$) in the Navier-Stokes equation⁴⁶⁻⁴⁹:

$$\rho (\partial \mathbf{u} / \partial t) + \rho (\mathbf{u} \cdot \nabla) \mathbf{u} = -\nabla p + \nabla \cdot [\eta (\nabla \mathbf{u} + (\nabla \mathbf{u})^T)] - \sigma \kappa \delta_s \nabla \phi + \mathbf{F}_m \quad (1)$$

$$\nabla \cdot \mathbf{u} = 0 \quad (2)$$

where, $\mathbf{u}$, p , ρ , σ , κ , δ_s and ϕ are the velocity, pressure, density, surface tension, curvature, smoothed delta function (zero everywhere except at the interface) and the level set function respectively⁴⁶⁻⁵⁰. For the volume fraction C of the dispersed phase ($C=1$ represents dispersed phase), the weighted average interpolation was used to define the density ρ and the viscosity η using the following equations^{50, 51}.

$$\rho = \rho_{cp}(1-C) + \rho_{dp} C \quad (3)$$

$$\eta = \eta_{cp}(1-C) + \eta_{dp} C \quad (4)$$

Magnetization of the magnetic Janus droplets

The nonlinear behaviour of magnetization of the magnetic Janus droplet at large magnetic fields can be described by the Langevin function $L(\gamma H)$ ^{46-48, 52}:

$$M(H) = M_s L(\gamma H) = M_s \coth \left[\gamma H - \left(\frac{1}{\gamma H} \right) \right] \quad (5)$$

where, $\gamma = (3\chi_0 / M_s)$. χ_0 and M_s are the initial magnetic susceptibility and the saturation magnetization, respectively. The magnetic susceptibility at an applied magnetic field H and for a given M_s , is defined by^{46-48, 52}:

$$\chi_H = \left(\frac{M_s}{H} \right) L(\gamma H) = \left(\frac{M_s}{H} \right) \coth \left[\gamma H - \left(\frac{1}{\gamma H} \right) \right] \quad (6)$$

Magnetic field and magnetic volume force

Maxwell's equations for magnetostatics were used to define the magnetic field⁴⁶⁻⁴⁸ by:

$$\nabla \cdot \mathbf{B} = 0 \quad (7)$$

$$\mathbf{B} = \mu \mathbf{H} = \mu_0 \mu_r \mathbf{H} = \mu_0 (1 + \chi_H) \mathbf{H} = \mu_0 (\mathbf{H} + \mathbf{M}) \quad (8)$$

where $\mu = \mu_0 \mu_r$ and $\mu_r = (1 + \chi_H)$ are the magnetic Janus droplet permeability and relative permeability, respectively.

The magnetic volume force $\mathbf{F}_m$ acts on the total volume of magnetic Janus droplet and determines droplet motion under a hybrid magnetic field $H^{51, 53}$. The force $\mathbf{F}_m$ for the dispersed phase volume fraction C , and susceptibility χ_H is given by the following equation,

$$\mathbf{F}_m = C \chi_H (\mathbf{B} \cdot \nabla \mathbf{B}) / \mu_0 \quad (9)$$

Simulation

The magnetic Janus droplet behaviour in a hybrid magnetic field was simulated by a DMMF numerical model developed from the above equations. COMSOL software was used to perform the simulations. The following Multiphysics modules (i) Laminar flow in microfluidic regime, (ii) Two-phase level set method and (iii) magnetic fields with no currents were utilized for simulations. The complete geometry consists of 89890 triangular elements. The microfluidic domain consists of 44138 triangular elements with average element quality of 0.9851 (size range: 0.16 to 15.3 μm). The magnetic field domain consists of 44728 triangular elements with average element quality of 0.9600 (size range: 3.75 to 500 μm).

Results and Discussion

Our studies, comprising magnetic Janus droplet control and fabrication of magnetic Janus particles can be categorized as (i) *Optimization of polymeric phase composition* to enhance protein binding, (ii) *Generation of magnetic Janus droplet*, (iii) *Magnetic Control of magnetic Janus droplet* for mixing and concentration of the magnetic component within Janus droplets, (iv) *Characterization of magnetic Janus particle*, (v) *Application for Protein Detection*. The following subsections describe our investigations in the above sequence.

Optimization of functional polymeric phase composition to enhance protein binding

The polymeric phase was optimized for protein binding by the addition of acrylic acid (AA) and ethylene glycol (EG) (**Figure 4**). Bradford assay was used as a rapid method for direct observation of protein binding. The optimum polymeric phase composition was determined in the following steps; (i) synthesize cylinders with 4 mm length, 1 mm diameter; (ii) perform EDC/NHS coupling protocol at a fixed concentration of BSA in PBS 1X buffer; (iii) wash thrice in PBS 1X buffer; (iv) add 0.5 ml of Bradford reagent; (v) wait 5 min and wash again thrice in PBS 1X buffer; (vi) observation of blue colour indicates extent of protein binding.

Figure 4: Optimization of polymeric phase composition. A: Direct observation of protein binding (a) PPS5, (b) 10 ml PPS5+2.5 ml acrylic acid, (c) 10 ml PPS5+2.5 ml acrylic acid + 2.5 ml ethylene glycol. B: Quantitative measurements of the mean gray value of (a-c). Acrylic acid and ethylene glycol were added to the polymeric phase to enhance protein binding. Bradford assay was used as a rapid method for visible and quantitative determination of protein binding from the developed blue colour. The decreasing mean gray value (or increasing contrast) denotes increasing amount of protein binding. The composition (c) was the optimized composition of the polymeric phase, as confirmed by the intense blue colour (lowest mean gray value) indicating higher protein binding compared to (a) and (b). Scale bar= 1 mm. Dimensions of the synthesized cylinder: 1 mm diameter and 4 mm length.

AA was added to the polymeric phase to enhance covalent bonding in the polymeric phase part of the magnetic Janus particle, which was found to increase BSA binding (Figure 4). The addition of ethylene glycol was also found to improve protein binding. The optimized composition of polymeric phase (Table 2) was found to be 10 ml PPS5+ 2.5 ml acrylic acid + 2.5 ml ethylene glycol, this composition resulted in significantly improved covalent bonding, increasing protein binding. 0.5 mg/ml rhodamine 6G was added to the polymeric phase to track mixing, concentration of the magnetic component during magnetic Janus particle synthesis and protein detection.

Generation of magnetic Janus droplet

To facilitate magnetic Janus particle formation, we first performed simulations for various dimensions of the flow focusing device as well as different sizes of microchannel outlet. To incorporate surfactant-free synthesis of Janus particle at high flow rates, the design was optimized for sufficient separation between magnetic Janus droplets (Figure 5). Droplets were generated by a flow focusing configuration under hybrid magnetic fields. Since capillary number (Ca) was less than 10^{-3} for the utilized experimental parameters, droplet formation is governed by the 'squeezing regime'. The capillary number⁵⁴ is given by $Ca = \eta_{cp} v_{cp} / \sigma$, where continuous phase viscosity, velocity, and surface tension are denoted by η_{cp} , v_{cp} , and σ , respectively.

The experimental and simulated sequence followed for magnetic Janus droplet formation in a flow focusing configuration is summarized as (a): (i) entry of dispersed phase, resulting in the formation of a paraboloid, (ii) increase in paraboloid size with increasing forward motion of the dispersed phase, (iii) formation of a neck as the paraboloid gets squeezed in the flow focusing by the flow of the continuous phase, (iv) neck elongation starts with progressive motion of the continuous phase and the dispersed phase, (iv) a critical point is reached when the paraboloid confines the continuous phase flow, resulting in 'squeezing' and thinning of the neck, (v) breaking of the neck and droplet formation.

Figure 5b shows the quantitative comparison between the simulated and experimental magnetic Janus droplet size. The performed simulations match closely with the experimental results.

Figure 5: Magnetic Janus droplet (MJD) generation. (a) Simulated MJD generation. Blue denotes the dispersed phase and red indicates the continuous phase of n-hexane. Refer Table 4 for the MJD set notations. Scale bar=500 μ m. (b) Comparison of experiments and simulations of variation of droplet diameter with flow rate ratio. Scale bar=250 μ m.

Magnetic Control of Magnetic Janus Droplets

Interesting behaviour was obtained when magnetic Janus droplet travels through the hybrid magnetic fields. The forward motion of the droplet results in circulation of each of the phases within the droplets in the opposite direction, resulting in mixing. This mixing is evident from the uniform colour of rhodamine in the droplets (Figure 6). At 0 mm, the uniform magnetic field acts on the magnetic nanoparticles (MNP), resulting in chain formation along the y direction. However, competition between magnetic and hydrodynamic forces determines the net force on the magnetic nanoparticles. At flow rate ratios less than 7.5, the magnetic volume force dominates, resulting in a greater chain formation. The non-uniform field component (H_{no}) of H acts on the magnetic nanoparticles with the highest value at $x=13$ mm, resulting in a net force on the magnetic phase. This concentration of the magnetic phase is significant at low flow rate ratios, viz., 2.5 and 5 (Figure 6(a-d)).

Since, significant concentration of magnetic phase was obtained at $x=13$ mm, synthesis of Janus particles was performed at the same location by focusing the UV beam at the point $x=13$ mm. The following subsections describe droplet micro-magnetofluidic control of the properties of the synthesized magnetic Janus particles.

Figure 6: Dynamic magnetic control of the magnetic phase in microchannels. (a-d) Magnetic Janus droplets (MJDs) generated at continuous phase flow rate of 1000, 2000, 3000, 4000 μ l/h, for a constant $Q_{wp}=Q_{pp}=200$ μ l/h. Refer Table 4 for the MJD set notations. Scale bar=250 μ m. Droplet flow is along the +x direction (blue arrow). The magnetic field is along Y direction (red arrow).

Role of the Hybrid Magnetic Field

We performed particle based simulations to demonstrate the role of the hybrid magnetic field on the concentration of magnetic component (brown dots) from the polymeric phase (blue dots) (Figure 7). To reduce computational requirement, shorter length of the channel was assumed. The hybrid magnetic field (H_o+H_{no}), with uniform magnetic field (H_o) region of 440 mT was then defined for 6 mm microchannel length. Simulations were performed with 200 particles for the magnetic component and 400 particles for the polymeric phase. Particle velocity corresponding to the experimental flow rates was then defined (Table 4, sets MJD1000 and MJD4000).

Simulations performed at zero magnetic field shows both phases (blue and brown dots) travelling together without any separation (Figure 7a), which matches with the experimental observations. Interestingly, when simulations were repeated with the applied hybrid magnetic field (Figure 7b, c), concentration of the magnetic component was observed. This concentration of the magnetic phase mainly occurred at the point of the highest gradient of the magnetic field. When magnetic Janus droplets containing polymer + magnetic component approaches near the point of highest field gradient,

the magnetic component gets attracted towards the magnetic field, resulting in the concentration of the magnetic phase. The uniform magnetic field zone (from -1 mm to 1 mm) do not contribute to the concentration of magnetic phase. Hence no change in the position of the magnetic component was observed when droplets travel from -1 mm to 1 mm. Droplets for $x \geq 1.5$ mm experience the field gradient H_{no} . This field gradient for $x \geq 1.5$ mm is directed towards the centre in the negative x direction, hence the magnetic component is pulled in the negative x direction. This magnetic force in the negative x direction results in the movement of the magnetic component in the negative x direction and contributes to magnetically controlled mixing inside the Janus droplet.

Figure 7: Particle based simulations of the effect of hybrid magnetic field at various times (in ms) on (a) MJD1000 at 0 mT, (b) MJD1000 at 440 mT, and (c) MJD4000 at 440 mT. Blue dots indicate polymeric component and brown dots indicate magnetic component. Purple arrow denotes the direction of the continuous phase flow. The concentration of the magnetic phase is evident from (b,c). Higher concentration of the magnetic phase can be observed at a lower flow rate of 1000 $\mu\text{l/h}$ (b) compared to the higher flow rate of 4000 $\mu\text{l/h}$ (c). Refer Table 4 for the MJD set notations. Scale bar = 400 μm . Insets (i–iii) show magnified images.

The highest concentration of the magnetic phase can be observed for $x > 2.5$ mm (640 ms for Figure 7b and 200 ms for Figure 7c), resulting in magnetic Janus droplet formation. At this point, Janus droplets are photopolymerized, leading to the formation of the magnetic Janus particles. Interestingly, the concentration of the magnetic phase for MJD1000 is higher compared to that of MJD4000, which matches the experimental findings (Figure 6 a, d).

Characterization of magnetic Janus particles

Synthesis of magnetic Janus particles was performed at $x=13$ mm, the parameters are summarized in Table 4. The following characterization was performed on the magnetic Janus particles.

Optical Microscopy. Optical microscopy analysis of the magnetic Janus particles was conducted. Figure 8(a–b) confirm the formation of the Janus particles, which is evident from the separated magnetic phase in the form of particle chains. Mixing of the polymeric phase and magnetic phase is evident from the fluorescent images in DF field mode with TRITC filter.

Figure 8: Optical micrographs of (a) MJP2500 and (b) MJP4000. BF indicate bright field imaging mode and DF TRITC indicates dark field imaging mode with TRITC filter. Refer Table 4 for the MJP set notations. Scale bar = 100 μm . The separated magnetic component is shown in the blue ellipse.

Vibrating sample magnetometer (VSM) Measurements. VSM measurements (Figure 9) were performed using a vibrating sample magnetometer (Lakeshore, model: 7400), for 10 to 15 mg of the synthesized magnetic Janus particles. All the synthesized samples exhibited soft magnetic behaviour with coercivity range of 4 Oe to 10 Oe.

Figure 9: Magnetic property measurements. Refer to Table 2 and Table 4 for notation. MJP2500 exhibited superior magnetic response compared to other samples. MJP5000 possesses high magnetic moment but was poorly polymerized due to higher magnetic phase content and smaller time in the polymerization zone. The inset shows coercivity in the range 4 Oe to 10 Oe.

As shown in Figure 9, the magnetic moment of the particles increases with the decreasing flow rate ratio (or increasing magnetic Janus particle size). MJP2500 demonstrated good

magnetic properties and good stability compared to other samples. MJP5000 shows high magnetic moment due to the higher dispersed phase flow rates. However, it possesses poor polymerization and hence, poor stability due to the high droplet velocity, resulting in reduced time in the polymerization zone.

FTIR Analysis. FTIR analysis revealed polymerization of synthesized magnetic Janus particle (Figure 10). Signature peaks at 1790 cm^{-1} , 1720 cm^{-1} , 1630 cm^{-1} , 1610 cm^{-1} , 1400 cm^{-1} and 800 cm^{-1} indicate the non-polymerized state of PEGDA samples, viz. PPS5, polymeric phase, and magnetic phase in liquid form⁵⁵. After UV polymerization, bands at 810, 1960, 2080, and 2880 cm^{-1} , corresponding to PEGDA either vanish or decrease significantly, indicating crosslinking.

Figure 10: FTIR analysis. Refer Table 2 and Table 4 for notations.

DSC Analysis. The DSC plots of samples are shown in Figure 11. The DSC plot corresponding to PEGDA reveals an endotherm at -20°C , attributed to its glass transition (T_g). An exotherm at 170°C corresponds to the crystallization temperature of PEGDA. The synthesized samples exhibited a T_g near 160°C , higher than that for PEGDA. This shift of T_g to higher temperatures is due to the presence of Fe_3O_4 nanoparticles. Poor polymerization of MJP5000 is evident from the DSC profile, which shows lower values of endotherms and exotherms compared to other samples. The MJP2500, MJP3000 and MJP400 samples exhibit similar DSC profiles. T_g for MJP2500 > MJP3000 > MJP4000, indicating a higher degree of polymerization of MJP2500.

Hence, superior polymerization was observed at lower flow rate ratios, corresponding to the set MJP2500.

Figure 11: DSC analysis. Refer Table 2 and Table 4 for notations.

Thermogravimetric analysis (TGA). Thermogravimetric analysis (Figure 12) was performed to determine the mass content of MNP. EMG 507 was used as the source solution of Fe_3O_4 magnetic nanoparticles.

Figure 12: Thermogravimetric analysis. Refer Table 2 and Table 4 for notations.

The TGA profile of EMG 507 exhibited 17% w/w of magnetic nanoparticles. Different liquid based phases, such as the polymeric phase and the magnetic phase, were utilized for the synthesis. The polymeric phase only contains polymers, which decomposes at 450°C . The magnetic phase contained 5.5% w/w of magnetic nanoparticles. Interestingly, all the synthesized magnetic Janus particles exhibited higher weight % of magnetic nanoparticles (8.4% for MJP2500, 7.8% for MJP3000, and 8.8% for MJP4000) compared to the magnetic phase, which is attributed to the effect of the hybrid magnetic field.

Hence, the use of the hybrid magnetic field for the synthesis of Janus structures separates the magnetic phase and increases the content of the magnetic phase in the Janus particles.

Application for Protein Detection

Application of these magnetic Janus particles (MJP2500) was demonstrated for protein detection. FITC-BSA at concentrations of 200 and 20 $\mu\text{g/ml}$, was used as a model protein. Covalent immobilization of FITC-BSA was performed by the EDC/NHS coupling protocol, described earlier in the *experimental methodology section*.

Figure 13(a-b) shows the fluorescent micrographs of MJP2500 after protein binding. Qualitative observation demonstrates higher fluorescent intensity at a protein concentration of 200 $\mu\text{g/ml}$ compared to the value at the concentration of 20 $\mu\text{g/ml}$. Quantitative analysis was performed by measuring the corrected total fluorescence per unit area (CTF/ μm^2) by ImageJ software. Measurements were repeated for ten different particles and averaged to obtain mean CTF. These mean CTF values were found to be ~ 120 and ~ 55 , at protein concentrations of 200 $\mu\text{g/ml}$ and 20 $\mu\text{g/ml}$, respectively, confirming good protein binding and detection capabilities of MJP2500.

Figure 13: (a-b) Fluorescent images of MJP2500 particles showing protein (FITC-BSA) binding, at protein concentrations (a) 200 $\mu\text{g/ml}$ and (b) 20 $\mu\text{g/ml}$. Scale bar=100 μm . (c) Quantitative measurement of protein binding, determined from the corrected total fluorescence intensity (CTF) per unit area (mean CTF or fluorescence/ μm^2).

In the earlier work, Janus particle synthesis was performed by separated phases. Our method of concentration of the magnetic phase from the polymer offers better control of particle orientation. These capabilities can be used to reduce the washing stage and the Janus particles can be used as individual platforms for detection, similar to a well in the 96 well plate. Hence, such magnetic Janus particles can be used to perform operations on a microfluidic platform, which are typically performed in a 96 well plate. The Janus particles were fabricated utilizing a simple, surfactant-free, wash-less, and rapid method by mixing, followed by concentration of magnetic component and finally photopolymerization of the droplets. Our approach can increase functionality, e.g., (i) chemical reactions can be performed just before photopolymerization and the resultant properties of the product can be observed in the Janus particles, (ii) antibodies dispersed in one phase can be mixed with the other phase prior to photopolymerization, and

(iii) a stable suspension can be used as one of the two phases to mix with incompatible polymers prior to photopolymerization.

Conclusions

A facile approach for the synthesis of Janus particles with wireless, programmable and remote control of droplets was demonstrated by combining a droplet micro-magnetofluidic methodology with hybrid magnetic fields. A capillary microfluidic platform was found to be advantageous due to its adaptability, flexibility and low cost. High flow rate droplet micro-magnetofluidic simulations were performed to optimize the geometry for surfactant-free, wash-less synthesis. The experiments and multiphysics simulations are in good agreement. The effect of flow rates and applied magnetic field on mixing, concentration of magnetic phase inside droplets, as well as magnetization, particle size and configuration of the magnetic component in magnetic Janus particle was investigated. Significant concentration of the magnetic phase was obtained at 400 mT for flow rate ratios less than 7.5 and continuous phase flow rates less than 3 ml/h. The magnetic field gradient contributed to the dominating effect of magnetic volume force, resulting in concentration of the magnetic phase. Magnetic Janus particle synthesis was performed at the point of highest magnetic field gradient to concentrate the magnetic phase after mixing. The functionality of the polymeric phase was enhanced for protein detection by the addition of acrylic acid and ethylene glycol. Control of the properties of magnetic Janus particles by droplet micro-magnetofluidic parameters was demonstrated. The application of synthesized Janus particles for protein detection was demonstrated, with BSA as a model protein. Quantitative analysis of the fluorescent intensity confirmed protein binding and detection.

Conflicts of interest

There are no conflicts to declare.

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