Development and characterisation of glucosamine sulphate magnetic nanoparticles for rheumatoid arthritis chemotherapy

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Rheumatoid arthritis (RA) is an autoimmune disease that principally attacks synovial joints. RA also cause inflammation in the joints and associated regions. There is an unmet medical need for RA as well as for the targetted drug delivery of anti-rheumatic drugs to improve its efficacy at a much lower doses while decreasing its side effects. Hence, in this Letter, the authors report the development and characterisation of glucosamine sulphate magnetic nanoparticles for magnetically targetted RA chemotherapy.

1. Introduction: Magnetic nanoparticles are used for several medical applications such as drug delivery, hyperthermia, subcellular compartmental isolation [1–9]. In addition, nanotechnology has been considered as a potential technology that can contribute for the clinical studies including disease prevention, detection, diagnosis, imaging and treatment. Several studies reported the widespread clinical use of nanoparticles especially targetted chemotherapy of various conditions [10–13]. One such example is the use of nanotechnology especially magnetic nanoparticles for rheumatoid arthritis (RA) targetted chemotherapy. RA is a chronic inflammation on joints that is an unmet medical need with no complete cure. In recent times, several research groups have shown that RA therapy only can improve the condition of the patient but are still not able to cure. Several RA patients have shown to have a deformity in the joints due to chronic inflammation progressively results in the destruction of the cartilage, bone, and ligaments [14]. Despite existing several treatments of RA that offers symptomatic relief, currently, there is no known drug that offers permanent cure. RA treatments must be two-folded that includes: (a) alleviating the current symptoms; and (b) limiting further destruction of the joints; otherwise will result in handicap if the disease is not treated at the appropriate time. However, such a manifestation never coexist together in a class of drugs, e.g. certain analgesics may alleviate the current symptoms but do not have any impact on the future destruction of the joints [15]. Clinicians use cortisone injections as a long-term treatment strategy. However, low doses of cortisone injections were shown to benefit if it is complemented with suitable anti-rheumatic treatment [16]. Drugs used for RA treatment are classified based on its application such as analgesics, anti-inflammatory and anti-rheumatic drugs. These drugs are administered to the RA patients either as tablets, ointments and intravenous solutions lead to poor bioavailability due to wide biodistribution. Huge drug doses are required to improve bioavailability causes unwanted side effects.

Recently, several drug delivery technologies have been developed for RA treatment that includes chemical-based drug modification, drug carrier-based delivery and use of matrices made of polymer for drug entrapment that are localised in desired bodily compartments. Furthermore, in order to alleviate RA conditions, it is essential to improve the drug therapy efficacy by adopting suitable drug delivery technology devoid of the above-listed limitations associated with conventional drug delivery systems.

Thus, there is a clinical unmet need for an appropriate drug delivery that can target and deliver the maximum drug at the target joints and to realise improved therapeutic effects. The potential use of magnetic nanoparticles for the magnetically targetted drug delivery is very well recognised recently. In this Letter, we report the engineering of magnetic nanocarrier for the delivery of anti-rheumatic drugs to achieve targetted as well as a controlled release for effective chemotherapy of RA. We report the formulation of glucosamine sulphate (GS) conjugated with iron oxide magnetic nanoparticles thereby to increase the bioavailability and half-life, further apply the magnetic nanoparticle externally to target the affected joints for effective chemotherapy of RA.

2. Materials and methods: GS was obtained as gift (15 gm, 99.7% purity) from M/s Sashan Pharmaceutical Pvt Limited, Pondicherry. Polyvinylpyrrolidone (PVP), hydroxy propyl methyl cellulose (HPMC), ammonia solution, sodium hydroxide and ethanol were purchased from LOBA Chemie Laboratory Reagents and Fine Chemicals, Mumbai. Ferric chloride and ferrous chloride were purchased from S.D Fine Chemicals, Mumbai. Sodium citrate and polyethylene glycol were purchased from Qualigens, Mumbai. All the above materials are of and used as analytical reagent grade.

2.1. Organoleptic evaluation: The organoleptic properties of the drug, polymer and other excipients were analysed visually and confirmed during preformlulation. The active substance GS was in the form of fine, white powder. HPMC was a greyish-white powder or granules. The polymer PVP was a creamy white, finely divided free-flowing powder. The magnetite was a black fine powder.

2.2. Fourier-transform infrared spectroscopy (FT-IR) analysis: The FT-IR spectrum of substances recorded between 4000 and 400 cm^{-1} in an FT-IR 410 pc spectrometer was compared with the reference spectrum provided in USP confirmed the identity of the substances used. Potassium bromide pellet method was carried out. GS and potassium bromide were compressed under 10 ton pressure in a hydraulic force down to form a transparent pellet. Compatibility studies were carried out to study the possible interactions between GS and other inactive ingredients. The following list of excipients studied with FT-IR spectroscopy such as polymer, stabilisers and GS, magnetic nanoparticles (final formulation). The compatibility studies between drug, polymer and other excipients showed that there was no appearance of any new characteristic peak when compared with the standard spectrums, confirms that there were no chemical interactions between the drug, polymer and other excipients. [17].

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2.3. Synthesis of magnetic nanoparticles: A solution of the mixture of $FeCl_3$ and Fe_2Cl_3 with a molar ratio of 2:3 was prepared by co-precipitation method under aqueous ammonia as a precipitating agent until the pH value was titrated to 9.0 in 100 ml deionised water, under vigorous stirring, containing 1 g PEG4000 emulsifier. The assembly of Fe_3O_4 magnetic particles was observed based on the colour of the solution turning black. The sample was kept in a water bath at 50°C for 30 min and further maintained at 80°C for 1 h. Finally, the synthesised magnetic nanoparticles were magnetically separated and further washed and rinsed until the pH reached 7.0, and the samples were dried for 4 h at 60°C in a vacuum dryer [18].

2.4. Synthesis of GS magnetic nanoparticles (GSMN): GSMN were prepared using spontaneous emulsification coupled with solvent evaporation method. A total of 15% of HPMC (stabiliser) dissolved in 10 ml of deionised water. A total of 50 mg of GS (drug) and 40 mg of magnetite dissolved in 5 ml of deionised water separately. Then the drug solution is added dropwise to the above aqueous solution, kept in a magnetic stirrer. A total of 50 mg of PVP (polymer) was separately dissolved in 5 ml of ethanol. The above organic phase was added into the aqueous phase by a syringe under magnetic stirrer for emulsification and was maintained at room temperature for 4 h for solvent evaporation. The sample was sonicated using a probe sonicator (Sonics, Vibracell 750X, USA) at 25% amplitude for 15 min. After complete solvent evaporation and sonication, the solution was centrifuged at 10,000 rpm for 15 min. The obtained GSMN were dried in a vacuum oven at 35°C and stored in a refrigerator [19, 20].

2.5. Characterisation of GSMN

2.5.1. Surface morphological analysis: The surface morphology of the prepared GSMN was examined under scanning electron microscope (SEM) (MIRA3, Tescan, Czech Republic). The sample was coated with gold in an argon atmosphere under suitable vacuum condition by using ion sputter chamber. The coated sample was mounted on a screw-shaped stub using double side coated carbon adhesive tape and were examined at 20.0 kV accelerating voltage [21].

2.5.2. Particle size distribution: The mean particle size and sample size distribution were determined through dynamic light scattering experiments carried out on a high-performance particle size (Bluewave, Microtrac, USA) carried out in a backscattering mode.

2.5.3. Magnetic susceptibility: Magnetic susceptibility meter (MS2, Fugro, Australia) was used to determine the magnetic susceptibility of the magnetic nanoparticles. Before starting the experiment, the instrument was calibrated to zero. When the particles were kept nearer to the sensor displays magnetic susceptibility value for that particle.

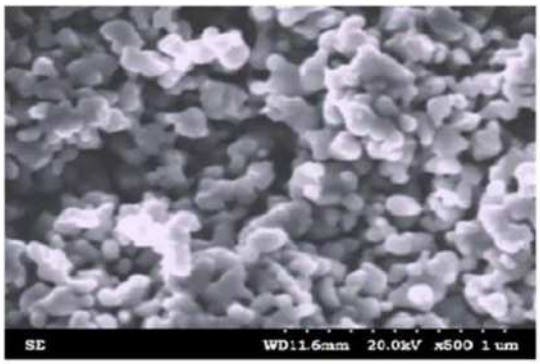
2.5.4. Targetting potential: A permanent magnet was placed at the sides of a glass vial and the time taken by the nanoparticles for complete accumulation towards the magnet was noted.

2.5.5. X-ray diffraction (XRD) studies: The crystallinity of the GS and magnetite was evaluated by using X-ray diffractometer (Rigaku-Miniflex Corporation, Japan) using Cu-Kα radiation ($\lambda = 1.5418$ Å) equipped with a scintillation counter detector at a voltage of 30 kV and a current of 15 mA with scanning angle 2θ ranging from 0° to 80°.

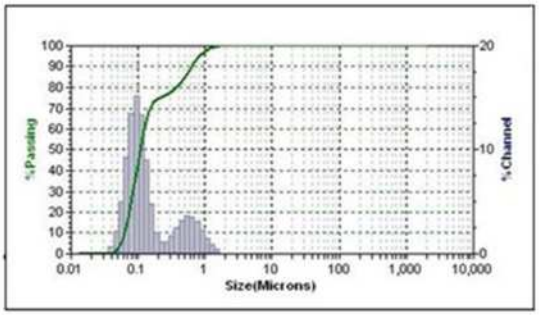
2.5.6. Construction of calibration curve of GS: A total of 25 mg of GS was dissolved in 40 ml of phosphate buffer (pH 6.8) and then made up the remaining volume to 50 ml with the buffer in a standard flask to produce the concentration of 50 µg/ml was used as a primary stock and used for the working standard preparation.

2.5.7. In vitro drug release of GSMN: A total of 150 mg of GSMN formulation equivalent to ~50 mg of GS was dispersed in a dialysis membrane bag, and then placed in 90 ml of phosphate buffer solution pH 6.8. The entire setup was kept at room temperature with constant magnetic stirring (250 rpm). At regular intervals of 5 min, 2 ml of the sample was withdrawn up to 420 min time intervals and simultaneously 2 ml of the buffer solution pH 6.8 was replaced into the same. The quantity of GS was determined by measuring the absorbance at 263 nm using UV–visible spectrophotometer (UV-1700 Pharmaspec, Shimadzu, Japan) [20–22]. The percentage of drug content was estimated from the calibration curve. Drug content (%) = Absorbance (Sample /Std) × Concentration (Std /Test) × 100.

Drug release kinetics studies: Release kinetics was determined by fitting the drug release versus time data in the zero order, first order, Higuchi equation, Korsmeyer–Peppas and Hixon–Crowell kinetic equations. (a) *Plot 1. Zero-order kinetics model*: Percentage drug to be released versus time which describes the systems where the drug release was independent of its concentration. (b) *Plot 2. First-order Kinetics Model:* Log percentage drug to be released versus time which describes the drug release from



a



b

c

Fig. 1 *Characterisation of GSMN*
a SEM images of GSMN
b Particle size distribution of GSMN
c Affinity of GSMN to an external magnet

the system, where the drug release was concentration-dependent. (c) *Plot 3. Higuchi classical diffusion kinetics model:* Percentage drug released versus square root of time which describes the system, where the drug released through diffusion from polymeric matrix. (d) *Plot 4. Korsmeyer–Peppas kinetics model:* Log percentage drug released versus log time. This is an exponential 'n' value is higher ($0.5<n<0.85$), then it will be non-Fickian diffusion or anomalous transport. This equation describes the mechanism by which the formulation loses its drug quantity with time either due to erosion or diffusion of the drug from the system. (e) *Plot 5. Hixson–Crowell kinetics model:* Cube root of percentage drug to be released versus time. In order to verify whether the drug released data was satisfying the above relationships, the plots were judged by the linear regression coefficient (R^2).

3. Results and discussion

3.1. GSMN synthesis: Magnetite nanoparticles were successfully synthesised by co-precipitation method was further conjugated with GS by using emulsification followed by solvent evaporation method yielded GSMN. The method for GSMN synthesis is very simple and facile.

GSMN characterisation: SEM images of GSMN are shown in Fig. 1*a* confirms the synthesised particles were homogenous and spherical present as agglomerates. The prepared GSMN were of the size range of <1 μm. GSMN particles are uniform in size, in a spherical shape. However, few GSMN particles were found clustered due to ionic properties of magnetite. Mean particle size of GSMN was measured to be 226 nm with polydispersity index as 0.0710 (Fig. 1*b*) determined based on laser diffraction. This indicates that the GSMN formulation is homogenous and monodisperse. Magnetic susceptibility of GSMN was found to be 36×10^{-5} was further confirmed visually as the particles in aqueous suspension of GSMN were magnetically attracted towards the magnetic field within 5 min at the glass wall nearer to that magnet when a permanent magnet was placed on the sides of a glass vial (Fig. 1*c*). Thus, the formulated GSMN with satisfactory magnetic susceptibility has the desirable attribute and potential for magnetic drug targetting with the aid of an externally applied magnetic field.

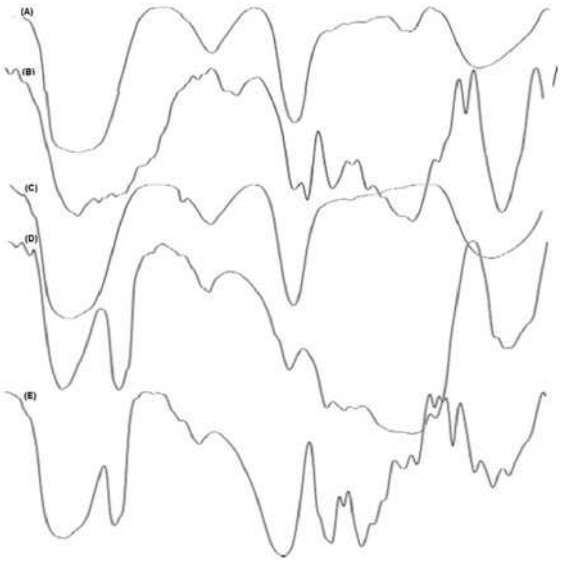


Fig. 2 *FI-TR spectrums*
A GSMN
B GS
C Magnetite
D HPMC
E PVP

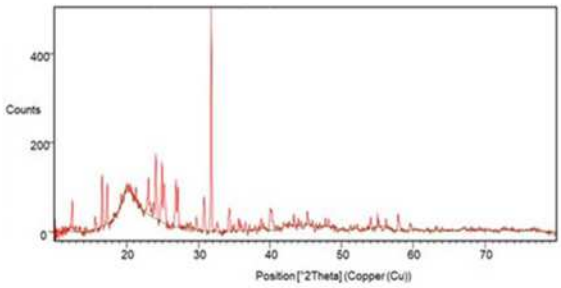


Fig. 3 *X-ray diffractogram of GSMN*

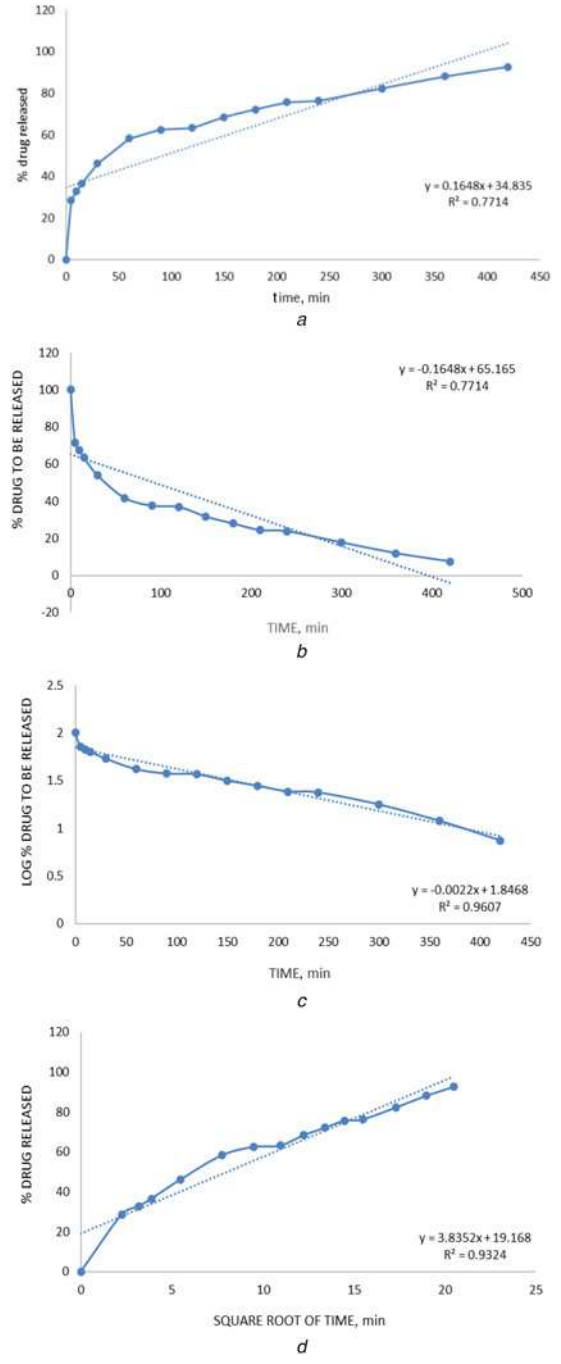


Fig. 4 *(a) Drug release profile of GSMN, (b–d) drug release kinetics plots for GSMN*
a In vitro drug release profile of GSMN at pH 6.8
b Zero-order kinetics plot
c First-order kinetics plot
d Higuchi kinetics plot

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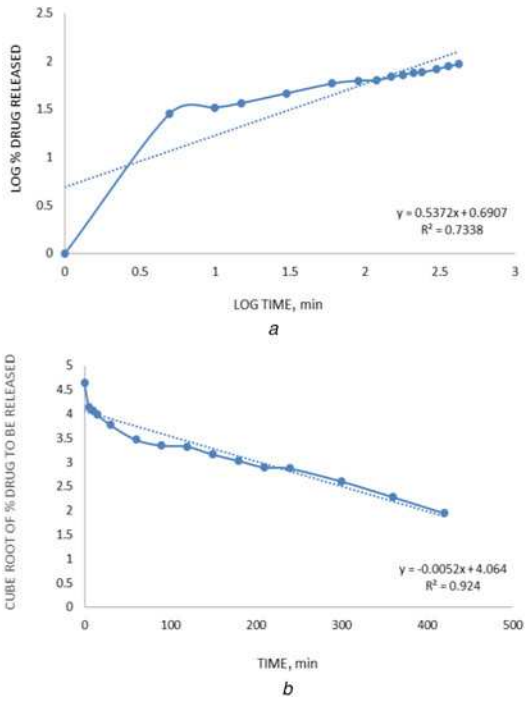

Fig. 5 *(a, b) Drug release kinetics plots for GSMN*
a Korsmeyer–Peppas kinetics plot
b Hixon–Crowell kinetics plot

The IR spectrum of GSMN shows absorption band at 3380.19 cm^{-1} indicates the presence of GS (Fig. 2*A*). The absorption band at 2077.54 shows the magnetite occurrence. The absorption band at 1638.23 cm^{-1} indicates HPMC. The presence of PVP is identified by the absorption spectrum at 1075.72 cm^{-1}. GS was identified by its significant strong absorption band for stretching at 1248.23 cm^{-1}. C–H bending was shown band at 1420.39 cm^{-1} (Fig. 2*B*). The FT-IR spectrum for magnetite (Fig. 2*C*) shows its characteristic absorption bands for Fe–O at 687 cm^{-1} and primary amines N–H bending vibration 1643 cm^{-1}, respectively. The FT-IR spectrum for HPMC (Fig. 2*D*) C–O–C shows its characteristic band at 1377 cm^{-1} along with C–O stretching gave absorption bands at 1052.60 cm^{-1}. Furthermore, 3480 cm^{-1} OH stretching, 2931 cm^{-1} C–H stretching alkanes are also observed. The FT-IR spectrum for PVP (Fig. 2*E*) shows primary amines N–H bending vibrations absorption band at 1628 cm^{-1}. The C–O formed its absorption band at 1081 cm^{-1}. FT-IR spectral analysis confirms the functional integrity of GS in the GSMN suggests that GS was physically entrapped in the polymeric matrix. No significant modifications in the spectral pattern confirms there is no chemical associations exist between the drug and the excipients. Hence, it is found that the drug GS in the GSMN formulation was compatible with magnetite (magnetic carrier), PVP (polymer) and HPMC (stabiliser) [21] X-ray diffractogram of GSMN shown in Fig. 3 confirmed the crystalline nature of GS and magnetite in GSMN. The minor deviations in the 2Θ observed perhaps is due to the physical association of the drug with the polymer.

Assay confirmed that GSMN contains 94% w/v of GS confirms the drug loading efficiency of the PVP polymer in association with HPMC as a stabiliser. In vitro drug release from GSMN is illustrated in Figs. 4 and 5. The drug release versus time profile was fitted to various kinetic equations in order to predict the drug release mechanism. It was observed that the drug release from GSMN obeyed the first-order kinetics and showed that the drug release was concentration-dependent. Similarly, the Korsmeyer–Peppas plot showed good linearity and the '*n*' value > 0.5, confirmed that the drug release was followed the non-Fickian diffusion or anomalous transport. From the above discussion the drug release from GSMN is governed by polymer solubility.

4. Conclusion: GSMN were developed by spontaneous emulsification followed by solvent evaporation coupled with ultrasonication. The formulation parameters were optimised-based preliminary trials in order to get better dissolution, better absorption, delay first pass metabolism, increase the half-life and eventually better bioavailability and drug efficacy. Since of the size of the magnetic carrier is being ~250 nm, phagocyte destruction of the formulated magnetic nanoparticles is negligible. Surface morphology is analysed by SEM and it is spherical.

The FT-IR studies clearly showed the presence of magnetite and the functional integrity of all other components without any unwanted chemical interactions. Similarly, XRD analysis confirmed the crystalline state of magnetite and GS. Magnetite, inherently possess superparamagnetic behaviour is a desirable attribute for the clinical applications of magnetic nanomaterials because zero-remanence magnetism will be realised upon removal of the applied magnetic field. Magnetic susceptibility data confirm the targetting ability of GSMN formulation in response to an applied external magnetic field. Due to the reduction in the particle size to nanometre range, the surface area increases and ultimately solubility and bioavailability enhances. Local bioavailability can also enhance at the target joints due to magnetic drug targetting. It is observed from the drug release profile, the prepared GSMN can release the drug in a controlled fashion over a period of 12 h at the target joints by restricting it with an external magnetic field. Drug release profile and release kinetics data confirms the controlled release of drug from the PVP polymeric matrix stabilised by HPMC present in GSMN. The prepared formulation was sufficiently stable over a period of 3 months when stored in room temperature. Thus, based on the observed results of the preliminary physicochemical, pharmaceutical and magnetic characterisation, the formulated GSMN looks promising as a potential nanocarrier for magnetically targetted drug delivery for RA.

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