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Research Article

Pharmaceutics for effective drug dosage

Synthesis and Evaluation of Multi Layered Magnetic Nanoparticles as Versatile Carrier for Anti-Cancer Drug Delivery

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Abstract: The objective of this study is to develop a passive targeting of multilayered nanoparticles encountering multiple obstacles on the way to their target due to the mucosal barrier, nonspecific uptake of the drug. To prevent the nonspecific drug toxicity and combination chemotherapy for synergistic effect, multifunctional ferromagnetic properties were successfully fabricated by the layer-by-layer assembly (LBL) technique. The drug-loaded magnetic chitosan nanospheres were coated alternatively with sodium alginate and chitosan up to 3 layers incorporated with melphalan and methotrexate. The core-shell type composites consisting of magnetic nanoparticles decorated with biological substances are interesting for various biomedical applications. The magnetic property investigation reveals that drug-loaded nanomaterials exhibit superparamagnetic behavior. The uniformly distributed magnetic nanoparticles were also observed in scanning electron microscopic images to characterize the synthesized product. The thin film consisting of chitosan nanospheres was coated alternatively with sodium alginate and magnetic nanoparticles had a conductivity of two formulations F1a and F2a were 35.2 emu/g and 43.4 emu/g respectively. The formulation was evaluated for its particle size, size distribution, zeta potential, and *in-vitro* drug release in the pH of 1.2 and 7.0 fitted in kinetic release studies. The developed magnetic nanoparticles showed promising results with better-delayed drug action and will be an enhanced therapeutic means in combating the infections of cancer therapy.

Keywords: Layer-by-layer assembly, magnetic nanoparticles, anticancer, magnetic drug targeting, methotrexate, melphalan.

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1. INTRODUCTION

Magnetic nanoparticles have many advantages like higher surface area, lower mass transfer resistance, easier to recover by a magnetic field.[1] Nanoparticles made of magnetic material can be used to concentrate agents at tumor sites using an externally applied magnetic field. Hyperthermia is a promising approach to cancer therapy. In magnetic fluid hyperthermia, magnetic particles act as the heating sources as the patient is exposed to an alternating magnetic field.[2] Magnetite nanoparticles can be made to accumulate only in tumor tissue, to overcome this obstacle.[3,4] The cancer therapy as well as tumor cell targeting therapy was established with minimum side effects, act directly against abnormal proteins in cancer cells termed targeted therapy.[5,6] Nanoparticles have emerged as a useful vehicle for poorly soluble agents such as Methotrexate and Melphalan. Chitosan and its derivatives are the most favorable macromolecules used to functionalize magnetic particles.[7] The solubility of the drugs in water is poor; however, it tends to be an intrinsic property of many drugs including powerful anticancer agents.[8] At the same time, intravenous administration of those intrinsically hydrophobic agents could be associated with serious safety problems. One of them is that the intravenous administration of relatively large aggregate/crystals of insoluble drugs that form in an aqueous media may embolize blood capillaries. Additionally, the low solubility of hydrophobic drugs in combination with excretion and metabolic degradation often does not allow achieving therapeutically systemic concentration. As a result, many promising drug candidates do not enter further development because of solubility problems. Methotrexate is used to treat certain types of cancer of the breast, skin, head and neck or lung. It is also used to treat severe psoriasis and rheumatoid arthritis (add reference). Methotrexate anti-tumor activity is a result of the inhibition of folic acid reductase, leading to inhibition of DNA synthesis and cellular replication.[9] Melphalan inhibits tumor growth by cross-linking guanine bases in DNA double-helix strands- directly attacking DNA.[10] It is used for myeloma and ovarian cancer. On the other hand, there exists an interesting approach to assemble polyelectrolyte multilayer shells on drug particles with few nanometers wall thicknesses through a layer by layer self-assembling technique. A layer-by-layer assembling technique (LbL) is based on alternate adsorption of oppositely charged polyelectrolytes on to certain surfaces.[11]These polycation/polyanion multilayers could be built with the required composition. Here, we suggest a novel application for an LbL coating technology to make stable aqueous nanocolloids for poorly soluble drugs with a very high content of the active drug and controllable drug release rate. Synthesis of multilayer magnetic nanoparticles to produce targeted and sustained release action for two selected anticancer drugs can be synthesized by using chemical reactions under controlled conditions and homogenization.[12,13] At first, the polymeric core can be synthesized by polymerization process to form a nano-size core. Then, the polymeric core need to be altered by a chemical reaction in such a way to coat another polymer which is used for fabrication of shell. Then the magnetic material can be incorporated by in-situ chemical reaction and polymeric core removed by extraction with a suitable solvent. Then the selected anti-tumor drug can be incorporated by homogenization. After that, a layer of another polymer can be coated by chemical interaction between the magnetic nanoshell and polymer. Then, another selected anti-tumor drug can be added to the outer polymer layer. The drug which is present in the outer layer will release first followed by the drug present in the core will be released in sustained manner.[12]

2. MATERIAL AND METHODS

Methotrexate received as gift sample (5 g, 99.7% purity) from Glaxo SmithKline Pvt. Ltd., Mumbai. Melphalan received as gift sample (8 g, 99.85% purity) from Celon Labs, Hyderabad. Styrene (Kemphasol Chemicals, Mumbai), ferric chloride, ferrous chloride, sodium alginate were procured from LOBA Chemie Pvt. Ltd., Mumbai, and reagents were of Synthetic Grade. Chitosan from Sigma Aldrich Chemicals Pvt. Ltd. Ammonia Solution analytical grade was procured from SD Fine Chemicals, Mumbai.

2.1 Preparation of magnetic nanoparticles

2.1.1 Synthesis of polystyrene nanoparticles as templates by an emulsion polymerization method

Styrene monomer 20 g was added with 200 ml of water containing 0.5% of tween 20 and it was emulsified at 1000 rpm for 15 minutes to form stable oil in water emulsion. 1.5% w/w of potassium per-sulfate (polymerization initiator) to styrene was added into the emulsion and it was heated to 70°C under stirring at 1000 rpm for 6 hours.[14] After 6 hours polymerization, polystyrene nanoparticles formulation (F1) was separated by centrifugation at 7,000 rpm for 15 minutes and washed with water and alcohol for 3 times. The product was dried in a vacuum oven. Polystyrene nanoparticles formulation (F2) was formulated with 2.0% w/w of potassium per-sulfate to styrene by using the same method.[15,16]

2.1.2 Sulfonation of polystyrene nanoparticles

Sulphonation is the process, where the sulfonic group attached to the polymer to increase the hydrophilicity.[17] The 5g of polystyrene nanoparticles F1 and F2 were separately dispersed in 25 ml of 95% sulphuric acid at 40 magnetic stirrings for 24 hours.[14] Sulphonated polystyrene nanoparticles F1 and F2 were separated by centrifugation at 7,000 rpm for 15 minutes and alternatively washed with water and alcohol for 3 times. The product was dried in a vacuum oven.

2.1.3 Synthesis of polystyrene core/chitosan shell nano assembly

1 g sulfonated polystyrene nanoparticles F1 and F2 were mixed with chitosan, dissolved in 30 ml of acetic acid solution (2%v/v) and vigorously stirred for 2 hours.[18] Then the polystyrene core/chitosan shell nano assembly F1 and F2 were separated by centrifugation at 7,000 rpm for 15 minutes and washed with water 3 times to remove free chitosan.

2.1.4 Magnetization of core-shell assembly

1g polystyrene core/chitosan shell nano assembly F1 and F2 were soaked in 0.2M Ammonium hydroxide solution for 24 hours and then dispersed into 20 ml water.[19] 10 ml solution of 0.2M ferric chloride and 0.1M ferrous chloride in the molar ratio of 2:1 was added into the dispersion under magnetic stirring for

2 hours. Magnetic core-shell assembly of F1 and F2 were separated by using a bar magnet and washed with water three times.[20]

2.1.5 Preparation of magnetic chitosan hollow nanosphere

The synthesized magnetic polystyrene core/chitosan shell nano assembly F1 and F2 were alternatively washed with tetrahydrofuran and water three times to remove polystyrene core and kept in a vacuum oven for 12 hours before use.[21]

2.1.6 Drugs loading into magnetic chitosan hollow nanosphere

Methotrexate and melphalan anti-cancer drugs were chosen as model drugs and loaded differently as shown in Table 1. Magnetic chitosan hollow nanosphere was dispersed into 50 ml of specified medium containing anticancer drug(s) and it was stirred for two days by using a magnetic stirrer (500 rpm). Drug loaded chitosan nanosphere was separated by magnetic field.[22]

Table 1. ***Formulation of methotrexate/melphalan Nanoparticles***

Formulation	Anti-cancer drug(s)	Chitosan hollow nanosphere	Medium
F1a	Methotrexate-10 mg	F1 – 20 mg	pH 6.4 phosphate buffer
F1b	Methotrexate and melphalan each 5 mg	F1 – 20 mg	Melphalan dissolved in 20ml ethanol mixed with methotrexate dissolved in 30ml pH 6.4 phosphate buffer
F2a	Methotrexate-10 mg	F2 – 20 mg	pH 6.4 phosphate buffer
F2b	Methotrexate and melphalan each 5 mg	F2 – 20 mg	Melphalan dissolved in 20ml ethanol and mixed with methotrexate dissolved in 30ml pH 6.4 phosphate buffer

2.2 Sodium alginate and chitosan layer-by-layer coating of drug-loaded formulations

10mg of each formulation was coated with sodium alginate (SA) by stirring at 500 rpm with 20 mg of SA in a 20 ml aqueous solution for 30 min.[23] SA coated magnetic nano assembly was separated and washed with water two times. Subsequently, the formulation was coated with 20 mg of chitosan in 20 ml of 1% glacial acetic acid with stirring at 500 rpm and the uncoated chitosan was removed by washing with water and centrifugation at 7000 rpm. Chitosan coated magnetic nano assembly was further coated with SA by the same method. The resulting products F1a, F1b, F2a, and F2b contained three layers in the order of SA/ chitosan/ SA.[7]

2.3 Characterization

2.3.1 Morphology

The surface morphology of melphalan/methotrexate magnetic nanoparticles was examined under a scanning electron microscope (SEM) (JEOL, JSM 6360, Japan). The formulation was subjected to freeze-dry then resulting solid content was mounted into screw-shaped stubs using double-sided carbon adhesive tape. The samples were coated with platinum in an argon atmosphere under vacuum condition by using Ion sputter camper and then they were examined at 15000 V accelerating voltage.

2.4 Particle size and zeta potential measurements

2.4.1 Particle size

The average particle size and zeta potential of the formulation F1a and F2a magnetic nanoparticles were measured using Malvern Zetasizer.[24]

2.4.2 Polydispersity index

The polydispersity index was determined using non-invasive back scatter technology which allows samples measurement in the range of 0.6 nm - 6 µm, freshly prepared layer by layer magnetic nanoparticles (1000 µl) was placed in a folded capillary cell without dilution. The measurement was carried out using a 4MW He-Ne laser as light source at a fixed angle of 173°C.[25]

2.4.3 Zeta potential

Particle charge is a stability determining parameter in magnetic nanoparticles and are measured by electrophoresis and expressed as dielectrophoretic mobility (or) converted to the zeta potential (mV) together with the hydrodynamic size of particles in the formulation by photon correlation spectroscopy.[26] Zeta potential was measured using Malvern Zetasizer (MAL 000967), performed at 25 ± 0.10°C, samples appropriately diluted with water. A Smoluchowski constant F (Ka) of 1.5 was used to calculate the zeta potential values based on the observed electrophoretic mobility.

2.4.4 Fourier transform infrared spectroscopy (FT-IR)

Drug-polymer interaction was studied by FT-IR spectroscopy.[27] This spectrum was recorded for pure drugs and drug-loaded magnetic nanoparticles using (FT-IR 98400, Shimadzu, Japan; sample were prepared in KBr discs 92 mg sample in 200 mg KBr) scanning range was 400-4000 cm^{-1} and resolution was 2 cm^{-1}.

2.4.5 Magnetic susceptibility

Vibrating sample magnetometer (VSM, Lakeshore, Model 7410 at 83 and 300K) was used to determine the magnetic properties of magnetic nanoparticle formulations with an applied magnetic field 0-2T at room temperature.[28] The Magnetic susceptibility of the formulated magnetic nanoparticles was determined by using a handheld magnetic susceptibility meter (Fugro, MS-2, Australia) by keeping the formulation at a distance of 1cm from the sensor.

2.4.6 *In-vitro drug release studies*

10 mg of formulation F1a, F2a and 20 mg of formulation F1b and F2b was performed by dialysis method in an open-end tube sealed with dialysis membrane (Himedia Laboratories Pvt. Ltd., Mumbai, India (size 12 -14 kDa) were dispersed in 5 ml of phosphate buffer of pH 6.4 separately.[29] Magnetic nanoparticles (5 ml) was added into the dialysis tubing's size 9 (Medicell, UK) and samples of buffer (1 ml) were withdrawn at predetermined time intervals from the external release medium for a period of 6 hours and replaced by the same volume of fresh buffer to maintain sink condition. The absorbance of withdrawn samples was measured using a double beam UV-visible spectrophotometer at 260 nm and 302 nm. The amount of drug present in each aliquot was determined from a standard calibration curve. The data obtained from *in-vitro* release rate studies were fitted with various kinetic equations like Zero order, First order, Higuchi, Korsmeyar-Peppas and Hixson-Crowell equation.[30]

2.4.7 *Before multilayer coating*

10 mg of formulation F1a, F1b, F2a, and F2b before multilayer coating were dispersed in 5ml of phosphate buffer of pH 6.4 separately. The dispersion was tied in dialysis tubings size 9 and placed in 60 ml phosphate buffer of pH 6.4 and stirred at 500 rpm. The 0.1 ml of medium was taken at a different time interval and it was replaced by 0.1 ml of fresh medium. The total amount of drug(s) released was determined by using the corresponding UV assay method described in the analytical part.

2.4.8 *After multilayer coating*

10 mg of formulation F1a, F1b, F2a, and F2b after multilayer coating were analyzed for its release property as same as drug release study before coating. The sampling influence on the concentration of the drug(s) was corrected for each sample with the following mathematical formula

$$C_n = Cn\ '+ \sum C_{n-1} Vs/60$$

Where, C_n-Corrected concentration, C_n'-Measured concentration at 'n^{th} time, C_{n-1}-Measured concentration at 'n-1'th time, V_s-Sample volume, C_{n-1}-Measured concentration at 'n-1'th time and V_s-Sample volume

2.4.9 *Evaluation of reaction kinetics of polystyrene polymerization*

Polystyrene polymerization reactions were conducted by using different initiator concentrations concerning the monomer i.e. 0.01%w/w, 0.5%w/w and 1.5%w/w of potassium persulphate to styrene and all other parameters were kept as constant. The 5 g of styrene monomer was added with 100 ml of zinc oxide nanoparticle dispersion and it was emulsified at 1000 rpm for 15 minutes to form stable oil in water emulsion. Potassium persulfate (polymerization initiator) to styrene was added into the o/w emulsion and it was heated to 70°C under stirring at 1000 rpm. 0.1 ml of the reaction medium was taken at different time intervals and the total absorbance was noted at 260 nm and 302 nm.

3. RESULTS AND DISCUSSION

3.1 *Characterization (Surface morphology)*

The SEM photographs of Fe_3O_4 nanoparticles of the formulations F1a and F2a were shown in Figure 1.1 & 1.2. The surface morphology of the formulations was uniform and spherical. The particles of formulation F2a was comparatively larger than F1a as that of particle size report.[31]

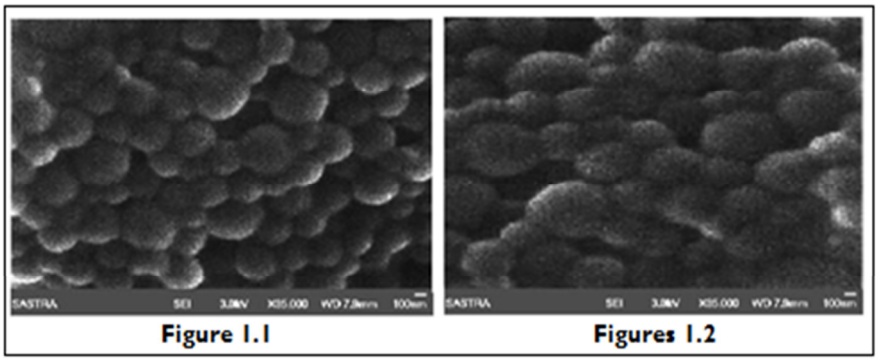

Fig 1. *The SEM photographs of Fe_3O_4 nanoparticles of the formulations F1a and F2a*

3.2 *Particle size analysis and size distribution*

The particle size analysis report is presented in Table 2. The particle size of the formulation F1a and F2a has increased after the coating of polyelectrolyte to the extent of 64.9 nm and 71.7 nm due to the deposition of an alternative layer of sodium alginate and chitosan. The particle size of the F1a was relatively small when compared to F2a. The reason for the smaller particle size of F1a is a lesser amount of initiator (1.5%) used to fabricate polystyrene templates when compared to F2a (2.0%). The Polydispersity index of the formulations before and after coating was found to be less than 0.6 which indicates the homogeneous distribution of particle.

Table 2. ***Particle size and polydispersity index***

Formulation code	Average Particle size diameter (nm)	Polydispersity index
F1a-Before coating	199.0	0.301
F2a-Before coating	212.4	0.577
F1a-After coating	263.9	0.230
F2a-After coating	284.1	0.306

3.3 Zeta potential measurement

The zeta potential of the formulations F1a and F2a was found to be -38.8 mV and -39.3 mV respectively (Figure 2.1 & 2.2). Nanoparticles that having the zeta potential in between +30 to -30 mV will tend to accumulate rapidly to form aggregates. The formulation of F1a and F2a having zeta potential less than the limit. So that the stability of the formulations was fine.

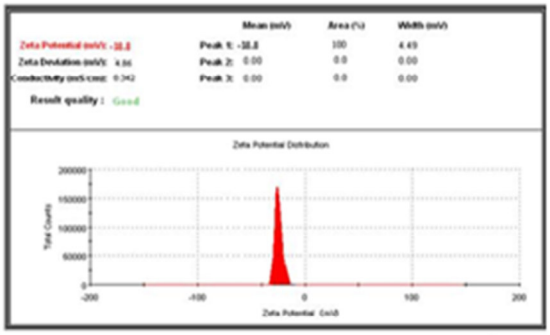

Figure 2.1

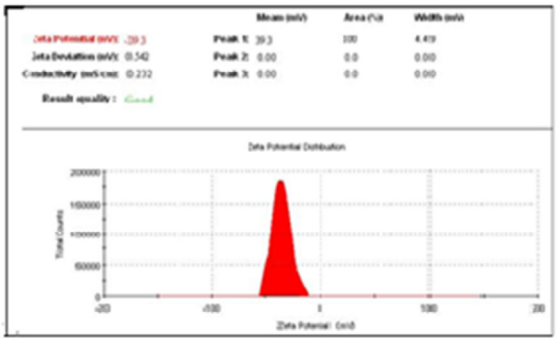

Figure 2.2

Fig 2. Zeta potential of the formulations F1a and F2a

3.4 Fourier Transform Infrared (FT-IR) Spectral characterization of the chitosan nanospheres

The FT-IR was used to illustrate the chemical reaction involved in the formulations. The FT-IR spectrum of sulfonated polystyrene displays a broad peak at 1593 cm^{-1} (amide bond), 3427.74 cm^{-1}(–OH bonded –NH absorption), 1380.11 cm^{-1} (methylene groups). 1597 cm^{-1} and 1384 cm^{-1} (C-C in phenyl group), 3398 cm^{-1} (C-H in phenyl group), 1597 cm^{-1} (C=C in aromatic ring), 2977 cm^{-1} (C-H in side chain). There were no peaks observed in the region 3100 cm^{-1} – 3000 cm^{-1} responsible for C=C alkene moiety. Styrene monomer contains C=C alkene moiety. Therefore, it was confirmed that styrene polymerized to polystyrene.

3.5 Nanocarrier compatibility with drugs

The FT-IR spectrum of a physical mixture of drug-polymer and compatibility of drugs melphalan and methotrexate. As there was no appearance of new peaks and significant changes in the spectrum pattern of the physical mixture which indicates the compatibility of drugs with polymers in the nanocarrier.

3.6 Magnetic susceptibility

The hysteresis curves (Figure 3) derived from the VSM analysis proved the superparamagnetic nature of the formulation F1a and F2a. The magnetic hysteresis loop is also evident for fine magnetic sensitivity to the applied external magnetic field. The magnetization values for formulation F1a and F2a were 35.2emu/g and 43.4emu/g respectively. Hence, it is possible to shift the nanoparticles along with drugs to the cancerous site by using an external magnetic field.

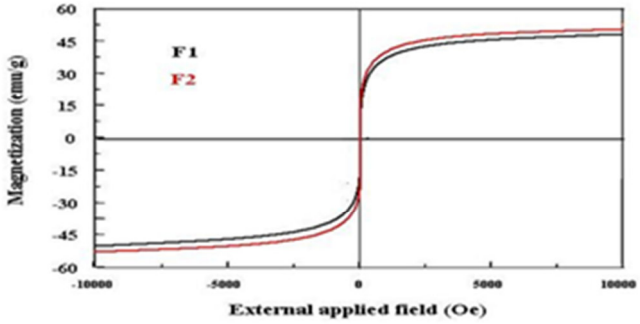

Fig 3. Hysteresis curve

3.7 *Drug entrapment efficiency and drug loading capacity*

The result of the drug entrapment and drug loading capacity study by UV and HPLC method was tabulated in Tables 3 and 4 respectively. The formulation F2a and F2b having more drug-loading capacity and entrapment efficiency compared to F1a and F1b for methotrexate and its combination respectively. The increased drug loading capacity of F2 is due to the comparatively large size than F1.

Table 3. Assay-UV method

Formulation	Absorbance	Amount of drug(s)/10 mg of formulation	Drug loading Capacity (%)	Drug entrapment efficiency (%)
F1a	0.345	0.5847	5.847	11.75
F1b	A_1-0.600	Melphalan-0.3132	Melphalan-3.132	Melphalan-12.53
	A_2-0.291	Methotrexate- 0.2277	Methotrexate-2.277	Methotrexate-9.11
F2a	0.367	0.6200	6.2	12.40
F2b	A_1-0.654	Melphalan-0.2916	Melphalan-2.916	Melphalan-11.66
	A_2-0.363	Methotrexate- 0.2945	Methotrexate-2.945	Methotrexate- 11.78

Table 4. *Assay-HPLC method*

Formulation	Peak area		Amount of drug(s)/10mg of formulation		Drug loading Capacity (%)		Drug entrapment efficiency	
	A	B	A	B	A	B	A	B
F1a	1088370	-	0.58342	-	5.834	-	11.67	-
F1b	851990.40	1937376	0.2280	0.3150	2.280	3.150	9.12	12.60
F2a	1162144.80	-	0.6220	-	6.220	-	12.44	-
F2b	1101612.64	1790996.5	0.2930	0.2912	2.930	2.912	11.72	11.65

3.8 *Drug release study and drug release kinetics*

Methotrexate release from the formulations F1a, F1b, F2a, and F2b were 82.26% 72.60%, 78.8%, and 81.5% in 72 hours respectively. Melphalan release from the formulations F1b and F2b was 75.4% and 79.70% in 72 hours shown in Figure 4.1 and 4.2 respectively. The drug(s) released from the formulation appears as constant with time without any burst release.[32] This is due to the polyelectrolyte coating of the formulations. But a slight deviation from the constant release appears. Hence, the actual drug release mechanism involved in the formulation was evaluated by subjecting the data to different release kinetic models.[33]

Table 5. *Drug release kinetics*

Formulation/ drug name	Correlation coefficient value (r^2) value				Korsmeyer-Peppas model		Drug release mechanism
	Zero Order	First Order	Higuchi model	Hixson-Crowell model	'r' value	'n' value	
F1a/A	0.985	0.803	0.895	0.991	0.999	0.836	Non-Fickian
F1b/A	0.994	0.800	0.856	0.989	0.999	0.945	Non-Fickian
F2a/A	0.993	0.802	0.873	0.995	0.999	0.891	Non-Fickian
F2b/A	0.999	0.803	0.841	0.985	0.999	0.981	Non-Fickian
F1b/B	0.994	0.800	0.856	0.989	0.999	0.945	Non-Fickian
F2b/B	0.992	0.805	0.881	0.993	1	0.873	Non-Fickian

A-Methotrexate, B-Melphalan

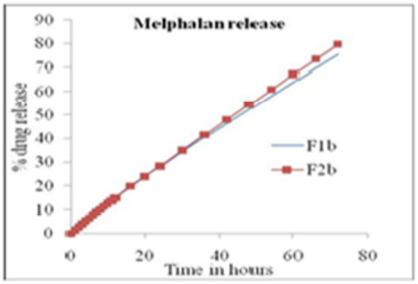

Figure 4.1

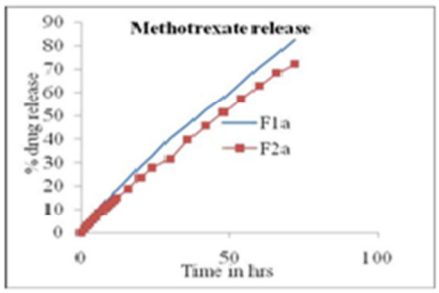

Figure 4.2

3.9 Drug release kinetics

The data derived from drug release study were subjected to various kinetic models and the results were summarized in Tables 6 and 7. The drug release kinetics of all the formulations obeyed Korsmeyer-Peppas model which indicates the diffusion-controlled drug release property.[34] The diffusion-controlled drug release may not obey Fick's law (Non-Fickian) due to many reasons. 'n' values derived from the slope of Korsmeyer-Peppas plot was used to identify the Fickian/Non-Fickian drug release. From the table calculated 'n' values found to lie between 0.8-1 which indicates the Non-Fickian type of drug release of the formulations. If 'n' value is 1, it indicates the zero-order drug release. It is possible to achieve zero-order drug release by optimizing the number/thickness of polyelectrolyte coating.[35]

Table 6. ***Drug release kinetics in methotrexate-F1a***

Time (t)	Amount Released (Q_t)	% Drug release	Square Log t	Root of time ($\sqrt{t}$)	Log Q_t	Fraction of drug release (f_t)	Log f_t	$(1-f_t)_{1/3}$
0	0	0		0	-	0	-	1
1	0.003534	1.2	0	1	-2.45173	0.012	-1.92082	0.995984
2	0.006984	2.371425	0.30103	1.414214	-2.15591	0.023714	-1.62499	0.992032
3	0.010471	3.555647	0.477121	1.732051	-1.98	0.035556	-1.44908	0.988005
4	0.013914	4.724476	0.60206	2	-1.85656	0.047245	-1.32565	0.983997
5	0.016923	5.74644	0.69897	2.236068	-1.77152	0.057464	-1.2406	0.980466
6	0.020205	6.860614	0.778151	2.44949	-1.69455	0.068606	-1.16364	0.976587
7	0.024746	8.40276	0.845098	2.645751	-1.60649	0.084028	-1.07558	0.971167
8	0.27754	9.424096	0.90309	2.828427	-1.55667	0.094241	-1.02576	0.967544
9	0.03063	10.40079	0.954243	3	-1.51385	0.104008	-0.98293	0.964054
10	0.033969	11.53453	1	3.162278	-1.46891	0.115345	-0.938	0.959971
12	0.040629	13.79609	1.079181	3.464102	-1.39116	0.137961	-0.86024	0.95172
24	0.191333	32.72326	1.380211	4.898979	-0.71821	0.327233	-0.39275	0.841175
60	0.413281	70.68261	1.778151	7.745967	-0.38375	0.706826	-0.15069	0.664317
72	0.482962	82.6	1.857332	8.485281	-0.31609	0.826	-0.08302	0.558277

Table 7. ***Drug release kinetics in Melphalan-F2b***

Time (t)	Amount Released (Q_t)	% Drug release	Square Log t	Root of time ($\sqrt{t}$)	Log Q_t	Fraction of drug release(f_t)	Log f_t	$(1-f_t)_{1/3}$
0	0	0		0	-	0	-	1
1	0.004082	1.4	0	1	-2.38908	0.014	-1.85387	0.995311
2	0.007799	2.674601	0.30103	1.414214	-2.10795	0.026746	-1.57274	0.991004
3	0.011502	3.94311	0.477121	1.732051	-1.93924	0.039443	-1.40403	0.986676
4	0.015161	5.199322	0.60206	2	-1.81927	0.051993	-1.28405	0.98236
5	0.01872	6.4197876	0.69897	2.236068	-1.72769	0.064199	-1.19247	0.978125
6	0.02236	7.668093	0.778151	2.44949	-1.65053	0.076681	-1.11531	0.973757
7	0.026233	8.996397	0.845098	2.645751	-1.58114	0.089964	-1.04593	0.969065
8	0.029432	10.09328	0.90309	2.828427	-1.53118	0.100933	-0.99597	0.965156
9	0.03284	11.26217	0.954243	3	-1.48359	0.112622	-0.94838	0.960955
10	0.036458	12.50259	1	3.162278	-1.43821	0.125026	-0.903	0.956456
12	0.04346	14.90395	1.079181	3.464102	-1.39191	0.149039	-0.8267	0.947625
24	0.08243	28.2683	1.380211	4.898979	-1.08391	0.282683	-0.5487	0.895166
60	0.195948	67.19747	1.778151	7.745967	-0.70786	0.671975	-0.17265	0.689661
72	0. 232405	7 9.7	1.857332	8.485281	-0.63375	0.797	-0.09854	0.587713

3.10 Polystyrene polymerization kinetics

The corresponding reaction time and the total absorbance values for 0.01%w/w, 0.5%w/w and 1.5%w/w of the initiator (potassium persulphate) were listed in Table 8. While plotting values in "X" and "Y" axis respectively as shown in graph 1a, 1b, and 1c, gives straight lines up. This indicates the polymerization reaction follows zero-order kinetics up to that particular time (Figure 5). Within this time around 99.5% of styrene was polymerized to polystyrene which indicates the end of the reaction. The reaction end time for polymerization of the concentration 0.1%, 0.5% and 1.5% were found to be 240, 80, and 20 minutes respectively. From the above result, it was concluded that while decreasing the initiator concentration it will increase the reaction time.[36]

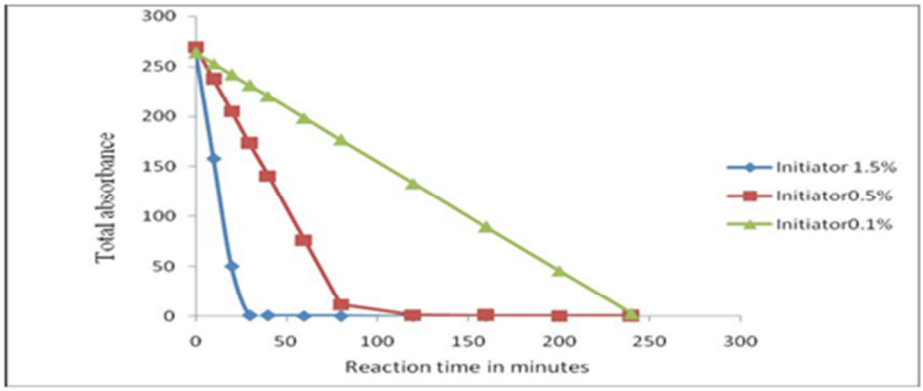

Fig 5. ***Polymerization kinetics***

Table 8. ***Polymerization reaction kinetics***

Reaction time (minutes)	Total absorbance		
	0.1%w/w initiator	0.5%w/w initiator	1.5%w/w initiator
0	263.8	269.5	265.1
10	253.0	237.2	157.6
20	242.0	205.0	50.10
30	231.2	172.7	0.652
40	220.2	140.5	0.425
60	198.4	76.00	0.198
80	176.6	11.50	0.064
120	133.0	0.979	0.010
160	89.48	0.536	0.001
200	45.90	0.324	0
240	2.320	0.064	0

4. CONCLUSION

In the last decade, the synthesis of MNPs covering a wide range of compositions and tunable sizes has made substantial progress. However, their effective utility has been limited due to higher particle size, loss of magnetization, other inherent properties and lower internalization capacity into the cancer cells which ultimately results in poor therapeutic efficacy in cancer treatment. Our present study illustrates layer-by-layer magnetic nanoparticles were synthesized by using sulfonated polystyrene as templates offer good stability. Anticancer drugs methotrexate and melphalan were combined successfully and loaded into the formulated magnetic carrier. The templates were coated with chitosan and consequently magnetized by ferric and ferrous chloride. The polystyrene core was removed to form the chitosan hollow sphere. Complete removal of the core is confirmed by the FT-IR spectrum of the magnetic chitosan hollow sphere. Drugs were then loaded into the magnetic chitosan hollow sphere and alternatively coated with sodium alginate and chitosan up to 3 layers to form layer-by-layer self-assembly. The particle size less than 300 nm showed significant accumulation in cancer sites but less than 150 nm will lead to phagocytic destruction. Hence, the formulations having the ability to accumulate into the cancer sites for a long time. Sustained release of drugs from nanostructured functional materials, especially MNPs, is attracting increasing attention because of the opportunities in cancer therapy and the treatment of other ailments. The potential of MNPs stems from the intrinsic properties of their magnetic core combined with their drug loading capability and the biochemical properties that can be bestowed on them using a suitable coating. In summary, we have synthesized magnetic nanoparticles via layer-by-layer assembly technique on sulfonated polystyrene as templates. Complete removal of the core is confirmed by the FT-IR spectrum of the magnetic chitosan hollow sphere. Surface morphology is analyzed by scanning electron microscope and it is spherical. The magnetic property investigation reveals that the magnetic nanoparticles exhibit superparamagnetic behavior.

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6. AUTHORS CONTRIBUTION STATEMENT

S.L. and P.S. designed and performed the experiments, derived the models and analyzed the data. T.P. and N.J. wrote the manuscript in consultation with S.L. and P.S.

7. CONFLICT OF INTEREST

Conflict of interest declared none.

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