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Pectin microspheres of curcumin coated with eudragit for novel drug delivery

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Abstract
The primary objective of this study was to develop and evaluate Eudragit-coated pectin microspheres (EU-MS) for targeted delivery of curcumin (CUR) to the colon. To achieve this, different ratios of CUR and pectin (ranging from 1:3 to 1:6), stirring speeds (ranging from 500 to 2000 rpm), and concentrations of emulsifier (ranging from 0.75 to 1.5% w/v) were employed to produce pectin microspheres (P-MS). The preparation yield and encapsulation efficiency (EE) of all P-MS were found to be high. The optimal formulation was determined to be MS with a drug:polymer ratio of 1:4, a stirring speed of 1000 rpm, and an emulsifying agent concentration of 1.25% w/v. The pectin microspheres were coated with Eudragit using the oil-in-oil solvent evaporation method, with a coat:core ratio of 5:1. The surface morphology, particle size and distribution, swellability, EE, and *in vitro* drug release in simulated gastrointestinal fluids (SGF) were evaluated for both the P-MS and EU-MS. The release of CUR from the EU-MS was influenced by the pH of the medium. In an acidic environment, the release rate was significantly slower, whereas at pH 7.4, the drug was released rapidly. Based on the findings of this study, it can be concluded that EU-MS show promise as controlled release carriers for delivering CUR specifically to the colon region.

Keywords:
Microspheres, curcumin, pectin, colon targeting, Eudragit coating.

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In the United States, colorectal cancer (CRC) is the second most common cause of cancer-related deaths, while the Indian subcontinent sees over 66,000 reported cases of colon cancer annually [1]. Traditional cancer chemotherapy is often ineffective in treating CRC due to the drug's inability to reach the intended site at a therapeutic level. Consequently, conventional treatment of colon cancer necessitates high doses of drug to compensate for the loss of drugs during their passage through the upper gastrointestinal (GI) tract. Unfortunately, these elevated doses can lead to undesirable side effects [2].

By delivering the drug molecule to the colon specifically, this problem can be solved. The creation of a site-specific delivery system that might regulate the delivery time for the active ingredient's release in the colon or the lower portion of the small intestine is a special difficulty in the pharmaceutical industry. Prodrugs, pH-sensitive polymer coating, and time-dependent formulations are methods for achieving drug administration in the colon [3]. The use of biodegradable polymers for colon targeting, such as azopolymer and polysaccharide (such as pectin and dextrin), is also documented in the literature [3]. The utilization of polymers that can be broken down by colonic bacteria is a promising approach for achieving targeted drug delivery to the colon. pH-dependent systemtakes advantage of the widely accepted understanding that the pH levels in the human gastrointestinal (GI) tract gradually increase from the acidic environment of the stomach (pH 2-3) to the slightly alkaline conditions of the small intestine (pH 6.5-7.0), and further to the colon (pH 7.0-8.0). The most commonly used pH-dependent coating polymers, such as methacrylic acid copolymers (specifically Eudragit L100-55, Eudragit L100, and Eudragit S100), dissolve at pH values of 5.5, 6.0, and 7.0, respectively. Pectin, on the other hand, is primarily a linear polymer consisting mainly of α-(1-4)-linked D-galacturonic acid residues with occasional 1,2-linked L-rhamnose residues [4]. A single pectin molecule typically contains several hundred to approximately one thousand building blocks. Since pectin readily dissolves in water, it is unable to effectively protect its drug content during transit through the stomach and small intestine.Because of their versatility in achieving a desired drug release profile, cost-effectiveness, and broad regulatory acceptability, hydrophilic polymer matrix solutions are frequently utilised in oral controlled drug delivery. The hydrophilic polymer matrices are particularly well suited for controlled-release applications because they can release a drug that has been trapped in an aqueous medium and control the release of that drug by controlling swelling and cross-linking. These matrices can be used to release drugs, charged solutes, and substances that are both hydrophilic and hydrophobic [5]. Numeroushydrophilic polymer matrices-based controlled release formulations have recently been created. Plant cell walls include a polymer called pectin. It is completely broken down by colonic bacteria, however the upper GI tract does not digest it. The solubility of pectin is a drawback. However, this flaw can be overcome by altering the level of methoxylation or by making calcium pectinate [6].

Materials and Methods

Materials

curcumin (CUR), a drug, was bought from HiMedia Laboratories Ltd in Mumbai, India, pectin was purchased. A gift sample of Eudragit S100 (EU) was obtained from SRL chemicals, Mumbai, India. We bought Span 85 from Sigma, Mumbai, India, along with acetone, isooctane, ethanol, hexane, and light liquid paraffin. Every other chemical employed was of the analytical reagent grade and was applied exactly as it had been.

Preparation of Eudragit-coated Pectin Microspheres (EU-MS)

The emulsion dehydration technique was employed to create the P-MS [7]. To achieve complete solubility, 3 g of pectin and 1 g of CUR were dissolved in 20 mL of distilled water and stirred overnight. This drug-polymer solution was then dispersed in 50 mL of isooctane containing 1.25% w/v of Span 85, while continuously stirring at 1000 rpm to form a stable water/oil (w/o) emulsion. The temperature of the solution was rapidly lowered to 15°C, and 50 mL of acetone was added to dehydrate the pectin droplets. Under mechanical agitation using a propeller stirrer at 1000 rpm, the system was maintained at 25°C for 30 min to ensure complete solvent evaporation. Subsequently, the microspheres were freeze-dried overnight using a Christ, Alpha1-2 LD plus, Germany, and stored in an airtight container for further analysis [8]. Pectin microspheres were prepared using different ratios of CUR to pectin, specifically 1:3, 1:4, 1:5, and 1:6.

ES was applied to pectin microspheres using the oil-in-oil solvent evaporation process. In order to achieve a 5:1 (coat:core ratio), pectin microspheres (each weighing 50 mg) were distributed in 10 mL of coating solution made by dissolving 500 mg of ES in ethanol:acetone (2:1). Then, 70 mL of light liquid paraffin containing 1% w/v Span 85 was added to this organic phase. To allow for solvent evaporation, the system was kept running at 1000 rpm at room temperature for 3 hours. The coated microspheres were then filtered, n-hexane washed, and overnight freeze-dried [8]. Using a laser diffraction particle size analyzer(Malvern Instruments Ltd., Malvern, UK) and distilled water, microspheres were suspended, and the software was used to determine the particle size and size distribution.

Scanning Electron Microscopy (SEM)

SEM was used to examine the surface morphology and form of P-MS and EU-MS. The formulation was sparingly sprinkled on a piece of double-sided tape that was fastened to an aluminium stub in order to prepare the samples for SEM analysis. The stubs were then coated with gold using a gold sputter module in a high-vacuum evaporator to a thickness of 300 under an argon environment [9]. Using a scanning electron microscope (Carl Zeiss Microscopy Ltd, EVO 18, Germany), photomicrographs of the coated samples were then taken after they had been randomly scanned.

Swelling ratio

The dissolution apparatus (United States Pharmacopoeia [USP] XXIII, Bio-Dis, Varian, Cary, CA, USA) was used to dissolve a known weight (100 mg) of various CUR-loaded P-MS and EU-MS in enzyme-free simulated intestinal fluid (SIF, KH_2PO_4/NaOH buffer, pH 7.4) for the required amount of time [10]. The microspheres were taken out and blotted with filter paper at regular intervals, and their weight change (after accounting for drug loss) was measured until

equilibrium was reached. The following formula was then used to compute the swelling ratio (SR):

$$SR = \frac{\mathrm{wg} \times \mathrm{w0}}{\mathrm{w0}}$$

where SR stands for swelling ratio, wg is the final weight of the microspheres, and 0 is their initial weight.

Drug Entrapment percentage

The microspheres (100 mg) were digested for 12 hours in 10 mL of 4% pectinase solution. Using high-performance liquid chromatography (HPLC), the digested homogenate was centrifuged (Remi, Mumbai, India) at 3000 rpm for 5 minutes to determine the CUR content. The SPD-M10AVP Shimadzu diode array detector and a Luna 5 C18 column (260 ±5.50 mm) were used in the HPLC system (Prominence, Shimadzu, Jappan) for the analysis of the drug. Class M10A (Shimadzu, Kyoto, Japan) managed the system. Acetonitrile/acetate buffer with a pH of 4.4 made up the mobile phase in a ratio of 15:85. CUR was found at 425 nm with a detection limit of 20 ng at a flow rate of 0.8 mL/min [11]. The peak area and concentration were plotted along a straight line, and the equation for the line, y = 0.1153x + 0.0147 with a correlation coefficient of 0.9998, was discovered. The linearity was noted in the concentration range between 0.1 and 40 g/mL. Before and during the analysis, validation and calibration were carried out.

In Vitro CUR Release in SimulatedGastrointestinal Fluids(SGF)

Pectin microspheres with and without eudragit coatings were compared for in vitro drug release in SGF. The paddle method described in USP XXIII was used to conduct the drug dissolving test on microspheres. Accurately weighted microspheres (100 mg) were evenly dispersed throughout 500 mL of dissolving media (SGF) at 37 ± 0.5°C, the content was spun at 100 rpm. During the period of the drug dissolving research, ideal sink conditions were present. By changing the pH of the dissolving medium at various time intervals, the simulation of GI transit conditions was made possible [12]. Using 0.1 N HCl, the pH of the dissolving media was maintained at 1.2 for two hours. After bringing the pH to 4.5 with 1.0 M NaOH, KH_2PO_4 (1.7 g) and Na_2 $HPO_4.2H_2$ O (2.2 g) were added to the dissolution medium, and the release rate study was conducted for an additional two hours. The pH of the dissolving medium was raised to 7.4 after 4 hours with 0.1 N NaOH and kept there for the following 24 h. A pipette with a microfilter was used to remove the samples from the dissolving liquid at different time intervals. Using the HPLC technique, the rate of CUR release was examined. By substituting an equivalent amount of SGF, the receptor volume was kept constant. Based on average calibration curves (n = 6), it was determined the concentration of CUR in the samples. Three copies of each dissolution study were carried out.

Statistical Analysis

By utilising various drug:polymer ratios, the mean percentage of CUR released in SGF (at varying pH) on both pectin microspheres and Eudragit-coated pectin microspheres were generated and compared. The statistical significance was ascertained using the Student t test. Statistical significance was defined as a *p* value of .05.

Results And Discussion

Preparation of EU-MS

The emulsion dehydration process was used to successfully create CUR pectin microspheres. Scanning electron photomicrographs (Figure 1a) demonstrate the creation of microspheres that are uniform, have their surfaces cross-linked, and are almost spherical. Using the oil-in-oil solvent evaporation process with a coat:core ratio of 5:1, the pectin microspheres were coated with Eudragit S100. SEM photomicrographs of the coated microspheres revealed that they had a spherical shape (Figure 1b). The procedure was tuned to create microspheres with small sizes, a narrow size distribution, high drug loading efficiency, and controlled drug release at the pH of the colon by varying the stirring rate and emulsifier concentration. The average size of the pectin microspheres ranged from 25.11 ± 2.5µm to 30.87 ± 2.8µm, depending on the concentration of pectin used, which varied from 3% to 6% w/v. The % of EE was consistently around 71 ± 5% for all microsphere formulations. Among the different pectin concentrations tested, the highest efficiency in loading the drug was observed with 3% pectin (refer to Table 1). It has been previously reported by Mohamed et al., (2022) that an increase in polymer concentration leads to the formation of a more viscous dispersion, resulting in larger droplets and subsequently larger microspheres [13].

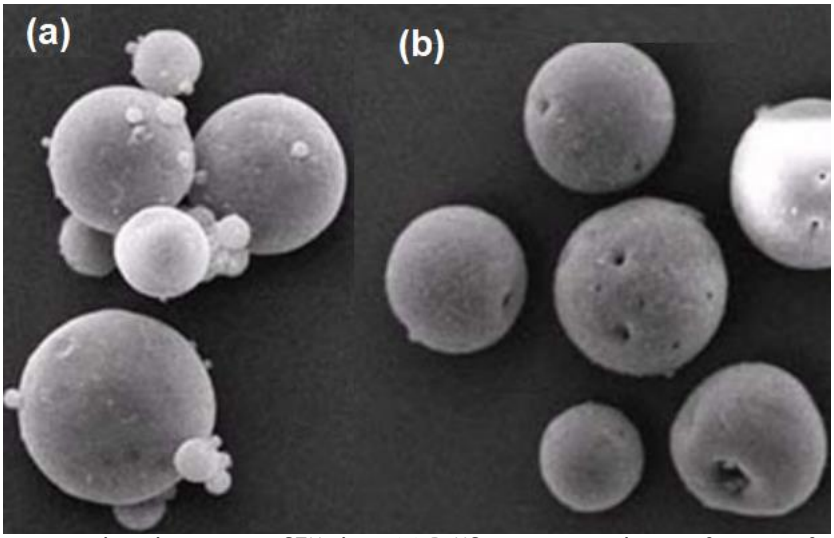

Figure 1. Photomicrographs taken using a SEM show (a) P-MS at an original magnification of ×420, and (b) EU-MS at an original magnification of ×3,000.

Furthermore, when investigating the effect of emulsifier concentration (Span 85) on microsphere formation, the average size of the microspheres ranged from 31.41 ± 1.9 to 23.63 ± 2.8 µm as the emulsifier concentration was varied from 0.75 to 1.5% w/v for the pectin microspheres.Particles with a smaller mean geometric diameter were produced as the surfactant concentration was increased. Smaller microspheres were produced as a result of stabilising the emulsion droplets and preventing their coalescence by increasing the concentration of Span 85 from 0.75 to 1.50% w/v. When making pectin microspheres, the drug loading efficiency varied from 68.22 ± 2.8% to 74.52 ± 3.1% with a range of emulsifier concentrations from 0.75 to 1.5% (Table 1). As the mechanical stirrer's agitation speed increased from 500 rpm to 2000 rpm, the mean diameter of the pectin microspheres fell from 30.13±1.4µm to 25.11 ± 2.1 µm. This outcome was anticipated since shearing forces required to split the oil phase into smaller globules are provided by high stirring speeds. The drug loading efficiency was found to be 69.21 ± 1.4% at 1000 rpm, which was determined to be the ideal stirring speed for pectin microspheres (Table 1).

Table 1. Effect of preparation Parameters on the Particle Size and Drug Entrapment Rate of Different Pectin Microspheres*

Specification	**Pectin Microspheres**		
	Preparation variables	**Averege Diameter (µm)**	**EE (%)**
Ratio of Polymer (Drug:polymer)	1:3	24.96 ± 1.9	73.95 ± 3.1
	1:4	27.11 ± 1.3	70.89 ± 1.6
	1:5	30.01 ± 1.2	65.05 ± 1.8
	1:6	29.99 ± 3.1	66.78 ± 3.3
Concentration of Surfactant (w/v)	0.75	31.41 ± 1.9	68.22 ± 2.8
	1.00	28.88 ± 1.3	68.51 ± 1.6
	1.25	26.41 ± 0.9	71.67 ± 1.9
	1.50	23.63 ± 2.8	72.95 ± 1.9
Stirring speed (rpm)	500	1.91 ± 1.1	69.21 ± 1.4
	1000	28.02 ± 2.0	68.09 ± 1.4
	1500	28.29 ± 1.8	73.55 ± 3.3
	2000	24.96 ± 2.6	74.52 ± 3.1

High stirring rates resulted in microspheres with an uneven form, but a slightly improved EE was detected. The optimal stirring time for pectin microspheres was found to be 30 min since [14], at this time, the microsphere size was small and the drug loading efficiency was good (74.52 ± 3.1%) (data not shown). Various microspheres' swellability was assessed with a greater resistance to swelling of EU-MS in the upper GI tract and a reduction in subsequent drug release at the nontarget location (Table 2) were ensured using EU-MS compared to P-MS [15].

Table 2. Degree of Swelling of Different Pectin Microspheres and Pectin Microspheres Coated with Eudragit*

S. No.	**P-MS**		**Eudragit-coated Microspheres**	
	Preparation Code (Drug:Polymer)	Degree of Swelling	Formulation Code (Drug:Polymer)	Degree of Swelling
1	P-MS1 (1:3)	0.91 ± 0.06	EU-MS1 (1:3)	0.05 ± 0.01

2	P-MS2 (1:4)	1.32 ± 0.12	EU-MS2 (1:4)	0.16 ± 0.02
3	P-MS3 (1:5)	1.39 ± 0.16	EU-MS3 (1:5)	0.18 ± 0.03
4	P-MS4 (1:6)	1.43 ± 0.15	EU-MS4 (1:6)	0.21 ± 0.03

In Vitro CUR Release

P-MS and EU-MS underwent an in vitro CUR release research in pH progression medium at 37 °C± 0.5 °C. The outcomes demonstrated that the polymer concentration had the biggest impact on the rate of CUR release from P-MS. P-MS released CUR in SGF in the following order: P-MS1>P-MS2>P-MS3 >P-MS4, as seen in Figure 2a. The disintegration of drug crystals on the surface of the microspheres may have caused the initial increased release of CUR from them [16].

The overall percentage of drug released from EU-MS exhibited the expected rate. No detectable drug release was observed within the first 2 hours in simulated gastric fluid (SGF) with a pH of 1.2. Similarly, at pH 4.5, the release of the drug CUR was quite minimal, accounting for only 2% up to 4 h [17,18]. The release of CUR from EU-MS in SGF followed the sequence EU-MS1 >EU-MS2>EU-MS3 >EU-MS4, as shown in Figure 2b.

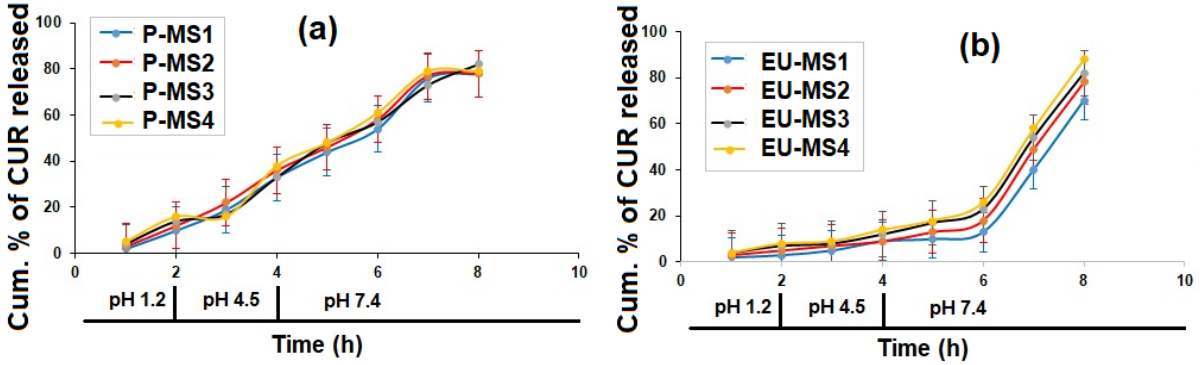

Figure 2. The cumulative percentage of CUR released *in vitro* from (a) P-MS and (b) EU-MS with varying drug-to-pectin ratios (ranging from 1:3 to 1:6) was measured in simulated gastrointestinal fluids with different pH levels. The reported values represent the average of three readings, with the standard deviation also provided.

Conclusion

When given in standard dose forms like tablets and capsules, the site-specific administration of curcumin from the system may lessen the negative effects of the drug brought on by its absorption from the upper section of the GI tract. The outcomes of the experiment showed that Eudragit-coated pectin microspheres might be used as a drug delivery vehicle for a successful colon-targeted delivery system. Further study required to establish the in vivo colon targeting for this formulation.

References

1. Mohamed JM, Alqahtani A, Ahmad F, Krishnaraju V, Kalpana K. Pectin co-functionalized dual layered solid lipid nanoparticle made by soluble curcumin for the targeted potential treatment of colorectal cancer. CarbohydrPolym. 2021; 252:117180.
2. Al-Serwi RH, Eladl MA, El-Sherbiny M, Saleh MA, Othman G, Alshahrani SM, Alnefaie R, Jan AM, Alnasser SM, Aishah E. Albalawi AE, Mohamed JMM, Menaa F. Targeted Drug Administration onto Cancer Cells using Hyaluronic Acid-Quercetin Conjugated Silver Nanoparticles. Molecules 2023, 28(10), 4146
3. Gahtani RM, Alqahtani A, Alqahtani T, Asiri SA, Mohamed JMM, Prabhu VS, Muluneh EY. 5-Fluorouracil-loaded PLGA Nanoparticles: Formulation, physicochemical characterization, and in vitro anti-cancer activity. Bioinorganic Chemistry and Applications 2023:2334675:1-11
4. Alamri, A.; Alqahtani, A.; Alqahtani, T.; Al Fatease, A.; Asiri, S.A.; Gahtani, R.M.; Alnasser, S.M.; Mohamed, J.M.M.; Menaa, F. Design, Physical Characterization, and Biocompatibility of Cationic Solid Lipid Nanoparticles with HCT-116 and 16-HBE Cells: A Preliminary Study. Molecules 2023, 28(4), 1711. https://doi.org/10.3390/ molecules 28041711
5. Srinivasan S, Elhassan GO, Janakiraman AK, Kayarohanam S, Dey T, JainafNachiya RAM, Nath UU, Mohamed JMM (2023). Preparation And Characterization Of Etoricoxib Ternary Complex For The Enhancement Of Solubility. Journal of Pharmaceutical Negative Results, 14 (2), 1703–1712.
6. Ahmad F, Mohamed JMM, Gayasuddin M, Anazi NA, Shaik RA, Humoud SYA, Ebrahim D, El-Sagheer AM, Amina Sabry AbdalghaffarEmam MSA. Molecular docking and In vivo gastroprotective effect of Salvia fruticosa. Research J. Pharm. and Tech. 2023, 16(1):314-322.
7. Saleh MA, Mohamed JMM, Ruby JJ, Kanthiah S, Alanazi YF, Majrashi KA, Alshahrani SM, Eladl MA, Alaryani FS, El-Sherbiny M, Menaa F. Preparation of Memantine-Loaded Chitosan Nanocrystals: In Vitro and Ex Vivo Toxicity Analysis. Crystals. 2023; 13(1):21.
8. Yadav K, Yadav D, Kumar S, Narra N, Mohamed JMM et al. Natural Biodegradable

and Biomass Polymeric Nanoparticles for the Delivery of Noscapine for cancer treatment. Biomass Conversion and Biorefinery, DOI: 10.1007/s13399-022-03334-y.

9. Mohamed JMM, Khan BA, Rajendran V, El-Sherbiny M, Othman G, Hussamuldin, ABA, Al-Serwi, RH. Polymeric Ethosomal Gel Loaded with Nimodipine: Optimisation, Pharmacokinetic and Histopathological Analysis. Saudi Pharmaceutical Journal, 30(11), 2022, 1603-1611.
10. Mohamed, JMM.; Alqahtani, A.; Menaa, F.; Kayarohanam, S.; Fatease, A.A.; Alqahtani, T.; Alamri, A.; El-Sherbiny, M.; Ramkanth, S.; Janakiraman, A.K. In Vitro Physical Characterizations and Docking Studies on Carvedilol Nanocrystals. Crystals 2022, 12, 988.
11. Mohamed JMM, Mahajan N, El-Sherbiny M, Khan S, Al-Serwi RH, Attia MA, Altriny QA, Arbab AH (2022) Ameliorated Stomach Specific Floating Microspheres for Emerging Health Pathologies Using Polymeric Konjac Glucomannan-Based Domperidone. BioMed Res Int. 2022: Article ID 3670946:1-12.
12. Al-Serwi RH, El-Sherbiny M, Kumar TVA, Mohamed JMM (2022) Molecular docking and Green Synthesis of Bioinorganic TiO2 Nanoparticles against E. coli and S. aureus. Bioinorganic Chemistry and Applications 2022, Article ID 30781146; https://doi.org/10.1155/2022/1142727.
13. Mohamed JMM, Ahamad F, El-Sherbiny M, Ebrahim HA, Eladl MA, Dawood AF, Khader STSA, Kavitha K, Teressa DM. An Ideal Approach for Enhancing 5-Fluorouracil Anticancer Efficacy by Nanoemulsion for Cytotoxicity against a Human Hepatoma Cell Line (HepG2 Cells). Biomed Res Int. 2022;2022:4094132.
14. Senthilvel CK, Karuppaiyan K, Pothumani A, Vedharethinam A, Jose AW, Mohamed JMM, Sherbiny ME, Ebrahim HA, Shafey ME, Dejene M. Development of Atorvastatin Calcium Biloaded Capsules for Oral Administration of Hypercholesterolemia. Evid Based Complement Alternat Med. 2022:2022:4995508.
15. Elhassan GO, Mohamed JMM. Development And In Vitro Evaluation Of Valsartan-Loaded Resealed Erythrocytes, International Journal of Applied Pharmaceutics, Vol. 14, 2022, 201-205, 10.22159/ijap.2022.v14ti.57
16. Alqahtani A, Raut B, Khan S, Mohamed JMM, Fatease AA, Alqahtani T, Alamri A, Ahmad F, Krishnaraju V. The Unique Carboxymethyl Fenugreek Gum Gel Loaded Itraconazole Self-Emulsifying Nanovesicles for Topical Onychomycosis Treatment. Polymers (Basel). 2022 Jan 14;14(2):325. doi: 10.3390/polym14020325
17. Mohamed JMM, Alqahtani A, Kumar TVA, Fatease AA, Alqahtani T, Krishnaraju V, Ahmad F, Menaa F, Alamri A, Muthumani R, Vijaya R. Superfast Synthesis of Stabilized Silver Nanoparticles Using Aqueous Allium sativum (Garlic) Extract and Isoniazid Hydrazide Conjugates: Molecular Docking and In-Vitro Characterizations. Molecules. 2021;27(1):110.
18. Mohamed JMM, Ahmad F, Alqahtani A, Alqahtani T, Krishnaraju V, Anusuya M. Studies on Preparation and Evaluation of Soluble 1:1 Stoichiometric Curcumin Complex for Colorectal Cancer Treatment. Trends in Science 2021;18 (24),1403