Preformulation Study Of Methotrexate Sodium (Mtx)

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ABSTRACT
Pre-formulation involves the use of biopharmaceutical concepts to determine the physicochemical parameters of a drug ingredient and excipients. The formulation design, manufacturing method, stability, pharmacokinetic qualities, and selection of container closure are all influenced by a range of physicochemical parameters. The main aim of the study is to conduct a preformulation research on methotrexate sodium in order to examine the drug's physicochemical characteristics and Creating, analyzing, and assessing PLGA-functionalized, methotrexate sodium loaded AuNPs (PLGA-MTX-AuNPs) for targeted and sustained administration and create, describe, and assess HA-MTX -AuNPs—methotrexate sodium loaded, hyaluronic acid functionalized AuNPs—for targeted and sustained administration and assess functionalized formulations utilizing flow cytometry and confocal imaging for cellular uptake and cytotoxicity studies, respectively, using MTT test

KEYWORDS: Methotrexate Sodium, HA-MTX, preformulation, methotrexate sodium

INTRODUCTION

The amine-capped ZnO quantum dot interacts with methotrexate (MTX) via a non-covalent contact, resulting in a significant enhancement in drug release. For our research, we selected MDA-MB-231, a kind of breast cancer cell known as triple-negative, which is resistant to methotrexate owing to its low levels of the MTX transport protein and RFC. To our knowledge, this is the first instance of a quantum dot being used as a nanocarrier to transport MTX to its resistant cancer cell MDA-MB-231 by non-covalent contact, without the need for any additional prodrug or covalent conjugation. In this context, pH acts as a natural catalyst for the dissolution of QDs at different pH levels, resulting in diverse drug release patterns. DDSs have pH-responsive behavior, resulting in the controlled release of a particular medication. These systems undergo changes in their chemical composition or physical structure when certain triggers are applied, resulting in a controlled release of medicines at the intended location. The tumor microenvironment exhibits distinct characteristics compared to normal cells, such as a lower pH range of 5.5–6.7 and higher temperature.

LITREATURE REVIEW

Al-Nemrawi, Nusaiba & Hameedat, Fatima (2022) A novel system was created by combining chitosan nanoparticles loaded with methotrexate (MTX-CS-NPs) and functionalized with photocatalytic TiO2 nanoparticles (TiO2-NPs). Upon exposure to UV light, this system is anticipated to trigger the breakdown of MTX-CS-NPs, leading to the release of MTX. The MTX-CS-NPs were synthesised and assessed for their particle size, charge, polydispersity, and drug release both before and after being coated with TiO2-NPs. An investigation was conducted to examine the excretion of MTX in a laboratory setting under conditions of darkness, normal light, and ultraviolet light. Subsequently, the in vitro evaluation of MTX-CS-NPs, both coated and uncoated, was conducted utilising the MCF-7 cell line. The functionalized nanoparticles exhibited increased size, greater polydispersity, and higher positive charges in comparison to the unfunctionalized nanoparticles. The entrapment efficacy exhibited a notable level of effectiveness, reaching 75%, and remained unaffected by the application of MTX-CS-NPs coating. In addition, less than 5% of methotrexate was released after 80 hours from uncoated nanoparticles, and the release was not improved by exposing the particles to UV light. On the other hand, the liberation of functionalized nanoparticles (NPs) was intensified, reaching a level of 40% after 80 hours, when the particles were exposed to UV radiation and when the quantity of TiO2-NPs utilised in the coating was increased. Coating the MTX-CS-NPs with TiO2-NPs ultimately increased their ability to kill MCF-7 cells. The cell viabilities of the coated MTX-CS-NPs were significantly lower compared to the other formulations. Ultimately, the drug release of MTX-CS-NPs may be activated and regulated from a distance by applying a covering of TiO2-NPs, potentially enhancing its efficacy in cancer therapy.

Kafle, Urmila & Agrawal, Satish & Dash, Alekha (2022) In 2020, breast cancer was the most prevalent kind of cancer, with 2.26 million cases and 685,000 fatalities reported. Untreated, this lethal illness may spread to remote organs, leading to its incurability, and resulting in fatalities. Presently, traditional treatments are used for the management of breast cancer; nevertheless, they exhibit many drawbacks including limited effectiveness in reaching the intended site, brief duration in the bloodstream, and unintended harm to non-targeted tissues. To tackle these difficulties, nanomedicines are the preferred option and are now undergoing thorough investigation for the treatment of

breast cancer. Nanomedicines are innovative methods of delivering drugs that may boost drug stability, solubility in water, duration in the bloodstream, controlled release, and targeted delivery to tumour sites. They also improve the safety and efficacy of therapy. Nanoparticles (NPs) may be delivered via many pathways. While the oral route is often chosen over the injection method for drug administration, the injectable route has many benefits. It allows for the customisation of medications with specific targets, increases the amount of drug delivered, bypasses the first metabolism in the liver, and enhances the pharmacokinetic properties of the active pharmaceutical components. Precise administration of nanomedicine, in close proximity to specific cellular structures like the mitochondria and nucleus in breast cancer, decreases the necessary dose and mitigates the harmful impacts of chemotherapeutic agents. This study is to provide the current state of recent advancements in several injectable nanomedicines used for precise treatment of breast cancer.

Mokhtar, Sarah & Khattab, Sherine (2022) The treatment protocol for some forms of breast cancer includes a combination of hormone therapy and chemotherapy. However, the effectiveness of this approach is restricted by the distinct pharmacokinetics of these therapeutic drugs, which hampers their simultaneous and targeted delivery to the cancer cells. In this study, we provide a hybrid carrier system that enables the simultaneous and targeted administration of the aromatase inhibitor exemestane (EXE) and methotrexate (MTX). The EXE compound was physically incorporated into liquid crystalline nanoparticles (LCNPs), while MTX was chemically linked to lactoferrin (Lf) by a carbodiimide process. The anionic EXE-loaded LCNPs were then coated with the cationic MTX–Lf conjugate via electrostatic interactions. The Lf-targeted dual drug-loaded LCNPs had a particle size of 143.6 ± 3.24 nm and a polydispersity index of 0.180. The drug loading demonstrated exceptional results, with an encapsulation efficiency of 95% for EXE and a conjugation efficiency of 33.33% for MTX. The combination of EXE and MTX had a synergistic impact against the MCF-7 breast cancer cell line, as shown by a combination index (CI) of 0.342. In addition, the Lf-targeted dual drug-loaded LCNPs exhibited enhanced and coordinated cytotoxic effects, as shown by a combination index (CI) of 0.242. Furthermore, the dose reduction index (DRI) values were 34.14 and 4.7 for EXE and MTX, respectively. Cellular uptake experiments revealed that Lf-targeted LCNPs exhibited greater cellular uptake into MCF-7 cancer cells compared to non-targeted LCNPs at both 4 and 24 hours. The targeted dual drug-loaded LCNPs show great potential as a candidate for combined hormone therapy and chemotherapy in the treatment of breast cancer.

Esim, Ozge & Oztuna, Ali & Sarper, Meral (2022) Methotrexate (MTX) is extensively studied for its therapeutic potential in treating many types of malignancies, such as breast cancer. Nevertheless, its use is restricted due to its disadvantages associated with elevated toxicity, inadequate solubility, and permeability, as well as a short plasma half-life. Hence, in this investigation, we proposed the utilisation of MTX-loaded innovative protein and polysaccharide-based nanovehicles to augment the efficacy of MTX in the therapy of breast cancer. We synthesised uncoated and chitosan-coated bovine serum albumin (BSA) nanoparticles loaded with MTX. The nanoparticles were characterised based on their particle size, dispersion, surface charge, encapsulation effectiveness, and morphological structure. In addition, we conducted a comparison of the cellular uptake, cytotoxicity, and ability to induce apoptosis between free MTX, MTX-loaded uncoated BSA nanoparticles, and chitosan-coated BSA nanoparticles on MCF-7 breast cancer cells. The application of a chitosan-coating caused a transformation in the surface charge of the uncoated BSA nanoparticles, increasing it from -25.5 mV to 53.3 mV. Consequently, the cellular absorption capabilities of the nanoparticles underwent considerable alterations in accordance with their surface charge. The chitosan-coated nanoparticles with a positive charge were internalised more effectively compared to the uncoated BSA nanoparticles with a negative charge. Furthermore, in accordance with investigations on cellular uptake, it was shown that chitosan-coated BSA nanoparticles exhibited a higher level of cytotoxicity and induction of apoptosis compared to both uncoated BSA nanoparticles and free MTX. The results revealed that the viability of MCF7 cells dropped to 58% and 35% after 48 hours of treatment with 5 μg mL^{-1} dosage of MTX using uncoated and coated BSA nanoparticles, respectively. In addition, the use of chitosan-coated BSA nanoparticles resulted in apoptosis-related cellular death that was 3.67 times greater than that of the free medication, and 2.23 times higher than that of the uncoated nanoparticles. The results of our research indicate that the MTX-loaded chitosan-coated BSA nanoparticles we produced might be considered a new and effective approach for treating breast cancer.

Danafar, Hossein & Taromchi, Amir (2022) The primary approach in cancer treatment involves the use of cytotoxic medicines as the main type of chemotherapy. Nanocarrier-based chemotherapy has gained significant popularity as a treatment for cancer in recent times. Prodrugs, which are drugs chemically bonded to a polymer chain, may greatly improve the effectiveness of drug integration. The objective of this research was to create biodegradable mPEG-PCL copolymers for the purpose of delivering anticancer drugs, namely curcumin and methotrexate, in a manner that is both effective and targeted. Additionally, the study sought to examine the physicochemical characteristics of these copolymers and evaluate their impact on breast cancer cells. The synthesis of mPEG-PCL copolymer was achieved by polymerizing dehydrated ὲ-Caprolactone monomer in the presence of dry methoxy polyethylene glycol as the initiator and Sn(Oct) 2 as the catalyst. The mPEG-PCL copolymers were synthesised and their structure was analysed using Fourier Transform Infrared Spectroscopy (FTIR). Subsequently, methotrexate was chemically linked to a copolymer. The MTX-mPEG-PCL, a compound of methotrexate and mPEG-PCL, underwent self-assembly into micelles when curcumin was present using the nano-precipitation technique. The drug loading was then evaluated. The study examined the cytotoxic effects of methotrexate, curcumin, and nanoparticles conjugated with methotrexate and curcumin on MCF-7 cancer cell lines using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test. The dimensions of the mPEG-PCL-MTX and mPEG-PCL-MTX-CUR nanoparticles were determined using a zeta sizer,

yielding average sizes of 51.89 ± 0.46 nm (PDI = 0.127) and 88.21 ± 0.31 nm (PDI = 0.344), respectively. The nanoparticles exhibited a zeta potential of -4.22 mV. The FTIR tests demonstrated that methotrexate was chemically bonded to the copolymer. The drug release in nanoparticles was greater at pH 5.5 compared to pH 7.4.

REESRACH METHODOLOGY

Pre-formulation involves the use of biopharmaceutical concepts to determine the physicochemical parameters of a drug ingredient and excipients. The formulation design, manufacturing method, stability, pharmacokinetic qualities, and selection of container closure are all influenced by a range of physicochemical parameters. The objective of pre-formulation studies is to ascertain the properties of the medicine and excipients, and mitigate any issues that may arise during later phases of product manufacture. This eventually leads to a reduction in cost and time by facilitating the development of a high-quality product.

Precisely measured 100 mg of Methotrexate sodium was placed into a volumetric flask with a capacity of 100 mL, and then 25 mL of deionized water was added. The MTX was dissolved in the solution, resulting in a stock solution of 1 mg/mL when the volume was adjusted to 100 mL with deionized water. To get a Methotrexate sodium solution with a concentration of 100 µg/mL, 1 mL of a stock solution was diluted with 10 mL of deionized water. The UV Visible spectrophotometer was used to do a UV Visible scanning within the wavelength range of 200-400 nm. The process was conducted in a manner similar to the one described earlier for the calibration curve of MTX in phosphate buffered saline with a pH of 7.4, serving as the solvent.

DATA ANALYSIS

Preliminary tests of deionized water

pH measurement

The pH of deionized water was tested using a calibrated pH meter, and it was found to be neutral (Table 1).

Table 1 pH measurement of deionized water

Parameter	Result
pH	7.07

Conductivity test

The conductivity of a reference solution containing 0.1 M potassium chloride (KCl) and deionized water was determined. The KCl standard solution was used to calibrate conductivity equipment that provide direct readings in microsiemens or millisiemens. Potassium chloride (KCI) is an exceptionally stable salt that serves as a globally recognized benchmark for measuring conductivity. The measured conductivity of deionized water was determined to be 1.25 µSiemens/cm. The measured and documented values of conductivity are shown in Table 2.

Table 2 Conductivity measurement of deionized water

Solution	Observed conductivity (µSiemens/cm)	Reported conductivity (µSiemens/cm)
0.1 M KCl (Standard)	12.8×10^3	$[11.615\text{-}12.824] \times 10^3$ [At 20 – 25 ^{0}C] (197)
Deionized water	1.25	[1.1-1.3] [At 20 – 25 ^{0}C] (Conductivity of Water USP)

Organoleptic properties

An analysis was conducted on methotrexate sodium to assess its organoleptic properties. The findings are shown in Table 3.

Table 3 Organoleptic properties of Methotrexate sodium

Parameters	Observation
Colour	Yellow to brown colour
Odour	Characteristic
Physical State	Amorphous powder

Characterization and analysis of Methotrexate sodium

UV Visible characterization

The maximum absorbance of pure Methotrexate sodium in deionized water and phosphate buffer saline at a pH of 7.4 was observed at a wavelength of 303 nm. Figure 1 displays the UV spectra of Methotrexate sodium in deionized water, whereas Figure 2 shows the spectra in phosphate buffered saline at pH 7.4.

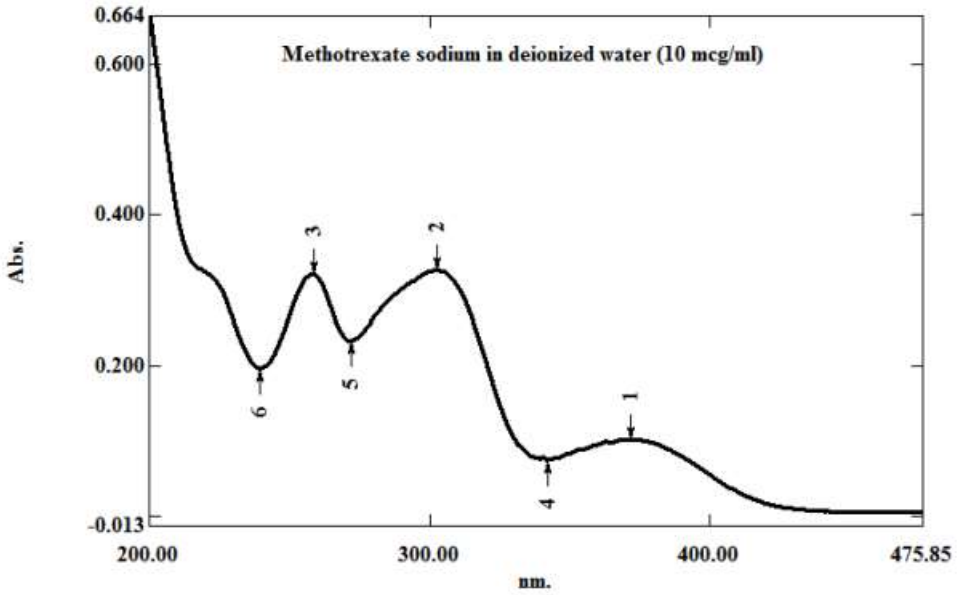

Figure 1 Absorbance maxima (λmax) of Methotrexate sodium in deionized water

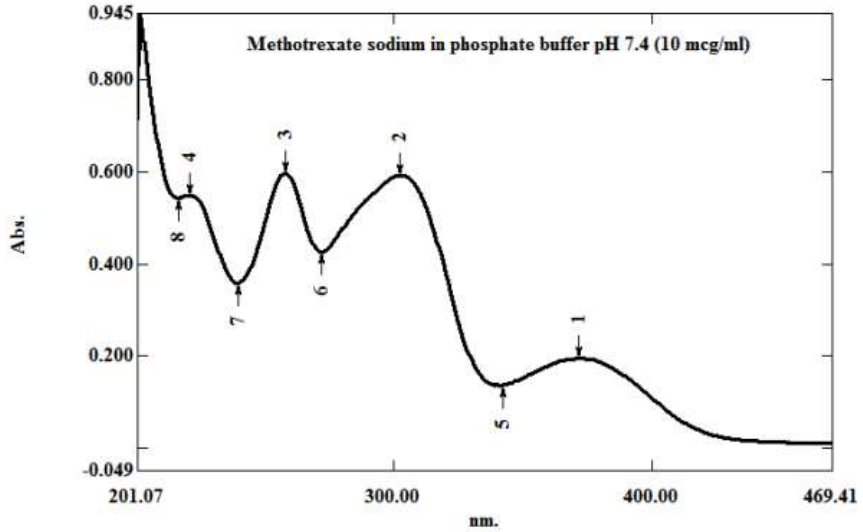

Figure 2 Absorbance maxima (λmax) of Methotrexate sodium in phosphate buffered saline pH 7.4

The UV Visible spectrum exhibited a peak at λmax = 303 nm, which corresponds to the typical UV Visible peak of Methotrexate sodium as specified in the Indian Pharmacopoeia (IP) 2010.

FTIR spectroscopy of Methotrexate sodium

Structure of MTX

Figure 3 depicts the molecular structure of Methotrexate sodium.

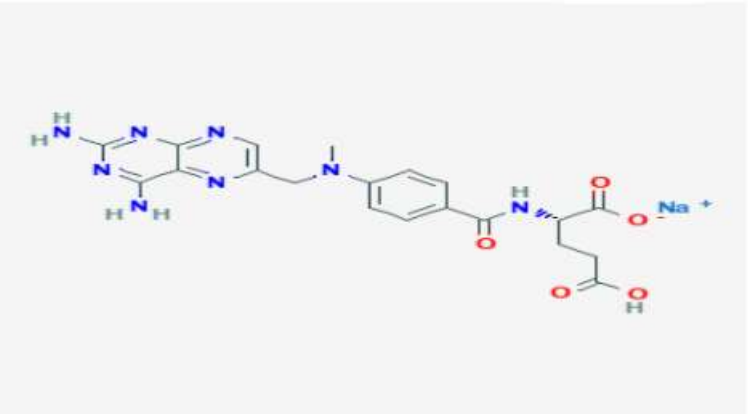

Figure 3 Structure of Methotrexate sodium

Figure 4 displays the typical dispersive infrared spectrum of pure Methotrexate sodium as provided in the International Pharmacopoeia, 8th edition, 2018.

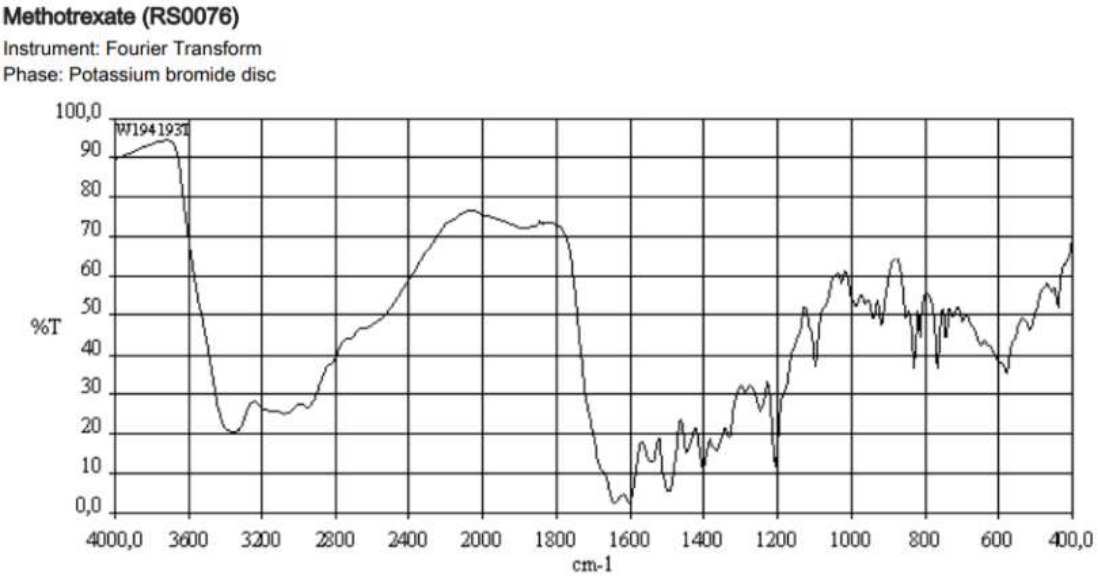

Figure 4 Standard dispersive IR spectrum of Methotrexate sodium

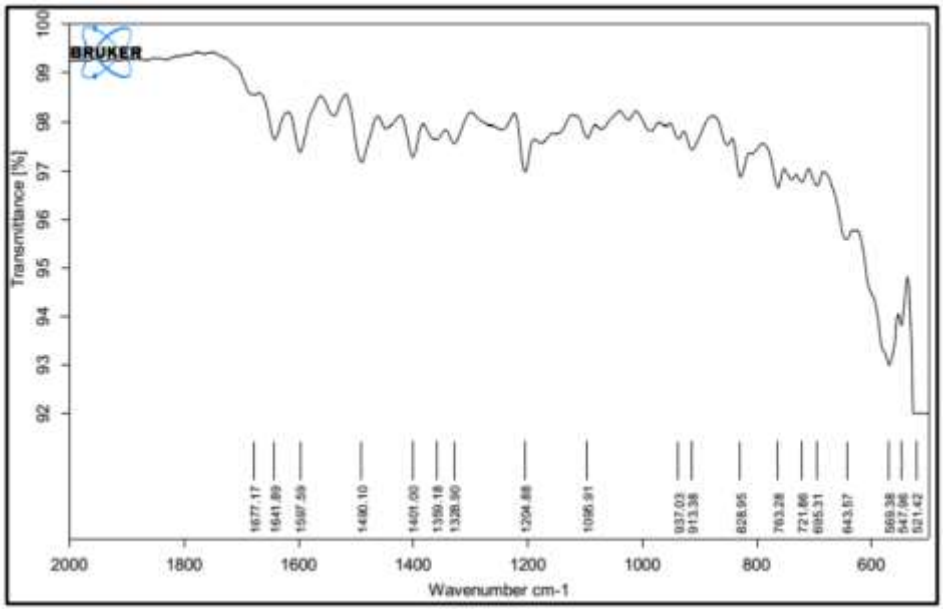

Figure 5 FTIR spectrum of sample Methotrexate sodium

The infrared (IR) spectrum of MTX was obtained using the FTIR Bruker alpha-E model, and the functional groups were analysed based on the molecular structure. Their appropriateness and structural compatibility with the drug and standard peaks of MTX, as specified in I.P. 2010, were confirmed. The Fourier Transform Infrared (FTIR) spectrum of Methotrexate sodium is shown in Figure 5 and detailed in Table 4.

Table 4 Characteristic peaks in the FTIR spectrum of Methotrexate sodium

Sr.no.	Functional group Observed	Sample curve Wave no. (cm^{-1})	Standard curve Wave no. (cm^{-1})
1	N-H stretch	3398.26	3100-3500
2	C=O stretch, -COOH	1639.81	1650 – 1600
3	C=C, aromatic ring stretch	1583.47	1615 – 1580
4	C-H, aromatic ring stretch	1455.33	1510 – 1450
5	C-O stretch, -COOH	1396.26	1440-1400
6	C-N stretch, aromatic primary amine	1347.81	1340-1250
7	C-N stretch, tertiary amine	1176.44	1210 – 1150
8	C-H, Substituted aromatic ring stretch	835.46	840-740

Differential Scanning Calorimeter (DSC)

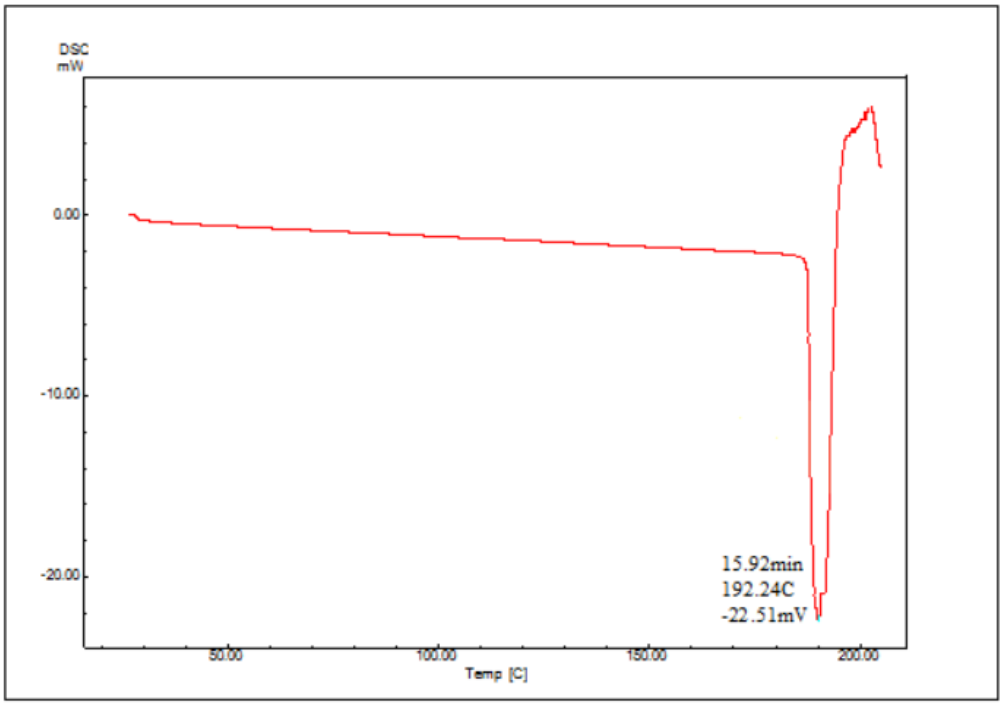

Figure. 6 DSC thermogram of Methotrexate sodium

Figure 6 displays the DSC thermogram of Methotrexate sodium. The thermogram shows that Methotrexate sodium displays an endothermic peak at 192.24 0 C, which corresponds to the drug's melting point.

CONCLUSION

Cysteamine was used in the research study to form a chemical bond between the –SH group and HA. The FTIR and NMR analyses confirmed the presence of all characteristic peaks, indicating the effective synthesis of thiol-conjugated HA. The formulation HA-MTX-AuNPs achieved a maximum drug loading of 29.55%. The particle size and shape of the formulation were verified using Transmission Electron Microscopy (TEM) and a zeta analyser. The conjugate HAMTX-AuNPs exhibited a hydrodynamic diameter of 81.75 ± 4.21nm, a polydispersity index (PDI) of 0.266, and a zeta potential of -32.8± 4.23 mV. The HA-MTX-AuNPs were examined using TEM and it was seen that a coating layer had developed on the surface of the AuNPs. The medication achieved a 90% release rate after 48 hours, indicating its sustained release properties. Therefore, the HA-conjugated AuNPs would be more efficacious in the therapy of breast cancer.

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