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Formulation, Characterization and Evaluation of Nanobubbles in Treatment of Rheumatoid Arthritis

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A B S T R A C T
Methotrexate (MTX), an orange-brown, crystalline and odourless compound with a melting point of 189°C, exhibits poor water solubility but shows improved dissolution in phosphate buffer. To enhance its delivery, MTX nanobubbles were formulated using Poloxamer 407 and polyvinyl alcohol (PVA). Characterization by XRD and FTIR confirmed the crystalline structure and chemical integrity of MTX. The resulting nanobubbles were small (23–185 nm), exhibited good stability (zeta potential ranging from -18.2 to -36.1 mV), and maintained a skin-friendly pH (~6.3–6.9). The formulations demonstrated high drug entrapment efficiency (60–89%) and sustained drug release for up to 48 hours, following the Higuchi diffusion model. Among the formulations studied, F3 and F4 showed superior performance, with F4 emerging as the optimal formulation. Stability studies confirmed the nanobubbles remained stable for six months. Overall, MTX nanobubbles offer a promising platform for sustained and controlled MTX delivery with potential applications in topical and systemic therapies.
Keywords: Methotrexate (MTX), Nanobubbles, Drug delivery, Poloxamer 407, Polyvinyl alcohol (PVA).

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C O N T E N T S

1. Introduction

Nanobubbles (NB) are nanoscale cavities filled with vapour or any gas in liquid and have received growing attention due to their high potential for applications in various scientific and engineering fields [1,2]. Nbs are either made by heterogeneous nucleation (within two interphases (solid/liquid/gas) or prepared homogeneously under atmospheric conditions in the presence of gas and may also be generated by the coalescence of vacancies in the diameter less than 1µm. Experimental studies report that nbs majorly form on a hydrophobic solid surface which can alter interfacial properties such as lubrication surface forces, adsorption and thus stabilize the colloidal particles. These nbs are experimentally produced by pressure release, heating, solvent exchange and water electrolysis [3-5].

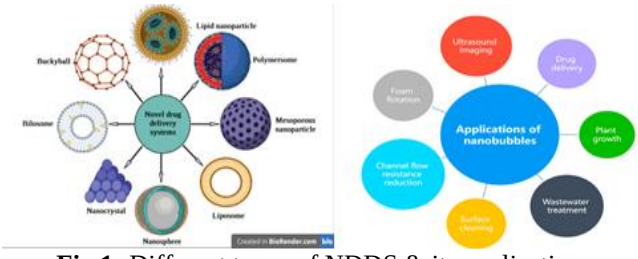


Fig.1: Different types of NDDS & its applications

2. Materials and Methods

Methotrexate (MTX) was sourced from IPC, India, and the marketed formulation Eptoin® from Abbott India Ltd. Chemicals such as acetonitrile, hydrogen peroxide, sodium hydroxide, hydrochloric acid, formic acid, niflumic acid, and phosphate salts were obtained from Spectrochem Pvt. Ltd., Thomas Baker Pvt. Ltd., RFCL Limited, and Sigma-Aldrich, USA. Rat feed was supplied by Pranav Agro Industries Ltd., Sangli, and bovine type II collagen by Chondrex, USA. Poloxamer 407 and monolein were procured from Gattefossé, France. Other materials like UPLC vials, dialysis membranes, polyvinyl alcohol, micropipettes, and 0.22 µm filters were purchased from standard suppliers.

Pre-formulation Studies

Physical appearance, melting point, solubility, partition coefficient, UV absorption, and FTIR (Fourier Transform Infrared Spectroscopy) studies were used to verify the medication sample's legitimacy. Furthermore, the compatibility of drugs and polymers was determined by comparing the FTIR spectra of pure drugs and polymers to the FTIR spectra of drug and polymer mixes.

Melting Temperature

MTX's melting point was determined using a digital melting point device. A narrow capillary tube was used, with one end of the tube shut. The material was poured to a height of 0.5 cm in capillary tubes. The sample-containing capillary tube was then put in the equipment' sample holder.

DSC Analysis

Accurately weighed sample was crimped on an aluminum pan and heated from 100 to 350˚C at a heating rate of 10˚C/min in air atmosphere. An empty sealed aluminum pan was used as reference

Solubility

In distilled water, ethanol and buffer solutions, the solubility of MTX was tested. To make a saturated solution, an excessive amount of MTX was dissolved in a measured amount of solvent and let to stand for two hours. UV absorbance at 303nm was measured after the solution had been filtered and diluted (Julio, et al., 2015).

Co-efficient of Partition

Shake flask method was used to calculate partition coefficient. In a separating funnel, accurately weighed medication was added to a mixture of N-octanol and water and agitated for 10 minutes. Overnight, the funnel was left to sit. UV spectrophotometer was used to test the different stages. (Kosiska-Szmuro et al., 2014; Cao et al., 2016).

X-RD Analysis

Small amount (1-2 mg) was weighed and spread over the sample holder. Sample holder was then kept into the XRD (Rigaku MiniFlex, USA) analysis chamber for analysis of phenytoin.

Preparation of Nanobubbles

Nanobubbles dispersions were made by emulsification of the cube-like lipid part comprising of MO and poloxamer 407 in water containing PVA 20 as appeared in table 4. The nanobubbles were shaped by softening MO and poloxamer 407 with the guide of hot plate kept up at 60 °C (MLH). At that point, the medication was dissolved down inside the melted blend. Distilled water containing 2.5 %w/w PVA (low viscous cube-like part is accomplished by utilizing natural solvents, preheated at indistinguishable temperature) was added to the melted blend underneath mechanical mixing at 500 rpm. Dispersions were kept up under blending at temperature for 2h to harden the lipid. The dispersions were exposed to mixing at 15000 cycles for each min. at 60°C for 1min (heidol pH homogenizers, silent gadget M and Germany). When the formulations were cooled, they were kept up at room temperature in glass vials.

Characterization of MTX-loaded nanobubbles

- Determination of PH
- Rheological Behavior
- Drug Excipients Compatibility Studies
- Shape And Surface Morphology
- Particle Size, Particle Size Distribution, Polydispersity Index (PDI) And Zeta Potential
- HPLC Method For MTX Quantification
- In Vitro Drug Release Studies
- Statistical Data Analysis
- Stability Studies

3. Results and Discussion

Physical characterization Identification of drug

Colour : Orange-brown
Nature : Crystalline
Odour : Odourless

Melting Temperature

MTX's melting point was determined using a digital melting point device with a narrow capillary tube with one end closed. The material was poured to a height of 0.5 cm in capillary tubes. The sample-containing capillary tube was then put in the equipment sample holder and the melting point of MTX was found to be 189ºC.

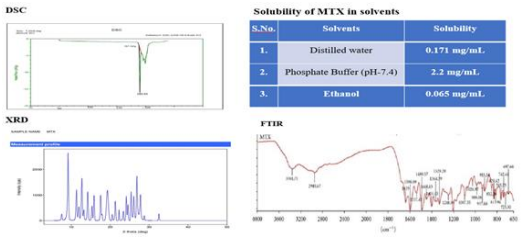
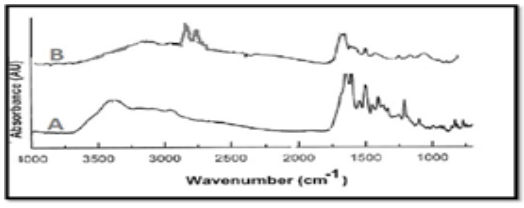


Fig.2: DSC, 2b: Solubility, 2c: XRD, 2d: FTIR



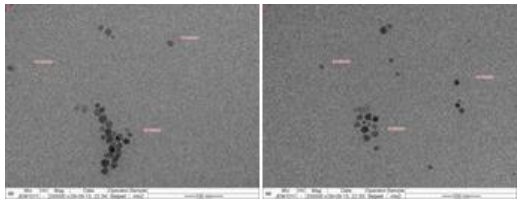


Fig.3: FTIR image showing A) MTX standard B) MTX loaded nanobubbles

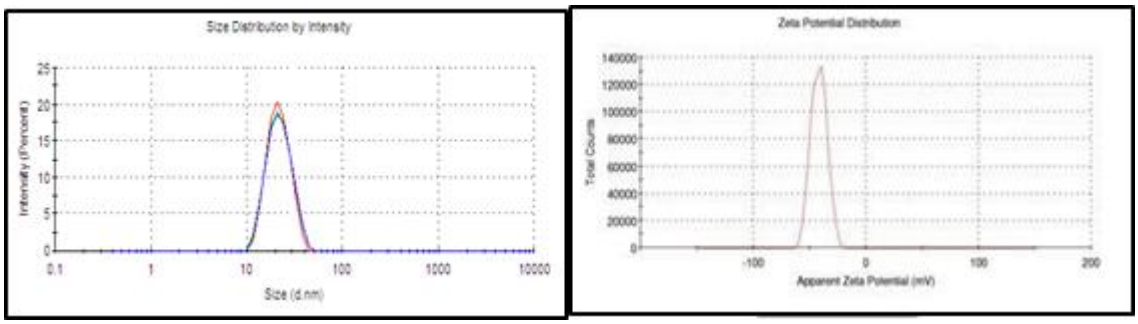


Fig.4: Particle size distribution and Zeta potential of nanobubbless

Table 1: Formulation

S.No.	Formulation Code	PLX:MO ratio	PLX (%w/w)	MO (%w/w)	Plx and MO: water
1.	F1	-	0.00	4.76	1:20
2.	F2	1:6	0.68	4.08	1:20
3.	F3	1:4	0.96	3.80	1:20
4.	F4	1:2	1.58	3.18	1:20
5.	F5	-	0.00	9.10	1:10
6.	F6	1:6	1.30	7.80	1:10
7.	F7	1:4	1.82	7.28	1:10
8.	F8	1:2	3.04	6.06	1:10
9.	F9	-	0.00	16.66	1:5
10.	F10	1:6	2.38	14.28	1:5
11.	F11	1:4	3.34	13.32	1:5
12.	F12	1:2	5.56	11.10	1:5

Table 2: Characterization of MTX nanobubbles

S.No.	Formulation	pH	Particle size(µm)	Zeta Potential(mV)	Viscosity(cp)	PDI
1	F1	6.62±0.01	185.32±12.10	-18.20±1.27	2.93±0.21	**0.754±0.03**
2	F2	6.41±0.05	93.90±3.90	-33.95±2.19	2.99±0.13	**0.391±0.02**
3	F3	6.88±1.10	68.32±1.32	-31.75±0.92	3.14±0.17	**0.427±0.03**
4	F4	6.71±1.40	23.21±0.89	-31.85±0.78	3.36±0.24	**0.124±0.03**
5	F5	6.42±2.60	154.11±11.94	-32.70±0.14	3.41±0.13	**0.652±0.05**
6	F6	6.32±1.90	168.75±3.72	-32.35±0.49	3.37±0.31	**0.331±0.03**
7	F7	6.68±2.40	143.56±10.94	-31.00±0.71	4.13±0.18	**0.215±0.06**
8	F8	6.94±1.30	130.71±8.35	-33.95±0.49	4.09±0.41	**0.391±0.02**
9	F9	6.65±1.9	121.32±9.72	-28.20±0.14	6.63±0.48	**0.731±0.03**
10	F10	6.39±2.4	78.21±5.47*	-33.60±0.28	73.91±4.3	**0.312±0.01**
11	F11	6.92±0.8	82.52±6.11	-36.10±2.97	79.97±4.9	**0.286±0.03**
12	F12	6.77±1.4	83.32±6.28	-32.70±0.99	84.32±5.3	**0.329±0.03**

Table 3: Entrapment efficiency and drug loading of Nanobubbles

S.No.	Formulation Code	Entrapment Efficiency%	Loading Capacity (%)
1	F1	64.39±0.94	11.22±1.34
2	F2	79.44±0.81	5.81±8.13
3	F3	80.31±0.72	6.41±9.28
4	F4	88.95±0.61	13.31±2.13
5	F5	60.39±0.24	12.01±0.36
6	F6	74.89±0.90	6.59±6.32
7	F7	77.62±0.53	6.46±0.29
8	F8	78.49±0.42	8.89±4.23
9	F9	60.99±0.94	10.92±2.19
10	F10	69.37±0.72	7.10±0.31
11	F11	70.63±0.44	7.52±7.32
12	F12	76.12±0.52	7.912.51

Table 4a: In-vitro drug release of Nanobubbless (F1-F6)

Time	F1	F2	F3	F4	F5	F6
1	2±4.32	2.7 ±4.56	3.2 ±1.36	4.8 ±10.9	1.7 ±5.46	2.4 ±4.32
2	7.3 ±3.21	8.1±6.31	8.3±2.87	15±5.25	6.9±8.14	7±2.14
4	20.6±2.13	19.7±2.14	22.3±7.13	30.2±4.31	17.7±4.63	17.9±4.69
8	28.9±0.13	24.7±8.32	30.3±7.13	31.2±2.33	31.9±4.63	19.09±4.69
16	49.7±11.32	51.3±4.32	53.1±8.36	58.9±7.43	42.3±7.98	45.4±9.32
24	57.9±2.13	60.4±9.41	63.4±4.37	73.7±2.62	55.4±1.02	54.6±4.85
48	78±3.18	84.3±5.32	88.1±8.43	88±3.13	75.3±5.36	76.2±3.28

Table 4b: In-vitro drug release of Nanobubbless (F7-F12)

Time	F7	F8	F9	F10	F11	F12	MF
1	2.4 ±1.25	2.9 ±4.23	1.9 ±5.87	2.1 ±8.33	3.1 ±4.27	3.4 ±5.61	25 ±3.9
2	7.1±5.34	6.9±2.36	6.6±4.51	6.9±7.59	7±4.59	7.2±7.79	49±4.19
4	18.2±9.32	18.8±3.59	16.4±3.19	17.5±9.08	18.3±3.61	17.3±4.69	92±1.29
8	20.2±4.12	21.8±3.59	16.4±3.19	17.5±9.08	18.3±3.61	17.3±4.69	92±1.29
16	47.5±2.65	47.6±7.59	43.7±9.75	44.3±5.46	46.7±5.01	48.2±5.68	92±2.3
24	56.3±6.59	56.7±9.78	52.9±8.32	53.1±6.32	58.2±2.32	58.9±7.34	92±5.32
48	79.1±5.43	78.9±5.98	73.9±4.56	74.3±8.11	74.8±1.14	58.9±9.12	93±1.23

Table 5: Stability Data

Condition	Time	Phase Separation	Precipitate	Particle Size ±SD	PDI ±SD	% Entrapment	% Drug Loading
Initial	0	No	No	78.45±0.42	-30.42±0.11	87.28±0.51	14.40±4.21
25±2°C/65±5 %RH	3M	No	No	69.32±5.31	31.26±0.48	85.72±4.36	13.37±3.91
	6M	No	No	95.18±1.42	30.77±8.22	83.37±0.42	13.04±6.31
40±2°C/ 75±5%RH;	3M	No	No	102.48±4.71	29.46±0.11	80.50±3.17	12.94±4.20
	6M	No	No	95.18±1.42	29.42±5.15	76.20±4.19	12.90±3.21

4. Conclusion

Methotrexate (MTX) nanobubbles formulated with Poloxamer 407 and PVA demonstrated excellent physicochemical characteristics, including small particle size (23–185 nm), stability (zeta potential −18.2 to −36.1 mV), and skin-compatible pH (~6.3–6.9). These nanobubbles exhibited high drug entrapment efficiency (60–89%) and sustained drug release over 48 hours, following the Higuchi diffusion model. Among the tested formulations, F4 showed the most favourable performance in terms of stability, entrapment, and controlled release. Furthermore, the nanobubbles maintained their stability for up to six months. Overall, MTX nanobubbles represent a promising strategy for sustained and effective MTX delivery.

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