Research Article

# Formulation and Evaluation of Metal Nanocomposite Microsphere Containing Methotrexate for the Treatment of Rheumatoid Arthritis

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**ABSTRACT**

Gold nanoparticle microspheres of Methotrexate formulation were formulated by spray drying technique, Nanoparticles were prepared using Gold chloride and tri sodium citrate by citrate reduction method and then the nanocomposite were prepared with chitosan solution which has further spray dried. All the prepared microspheres were evaluated for physical characteristics such as particle size, encapsulation efficiency, swelling ability, In-vitro drug release characteristics. Average particle size of microspheres was found to be in the size range 10-30 µm. All the prepared microspheres produced good controlled release of drug. From the study it is concluded that controlled release of methotrexate microsphere can be successfully prepared by spray drying method which can be administered by intra- articular route for the treatment of arthritis.

**Keywords:** Methotrexate; chitosan; Tri sodium citrate; Microspheres.

## INTRODUCTION

Nanotechnology is a field focused on the preparation of nanoparticles ranging from 1 to 100 nm, utilizing various synthesis strategies and modifications in particle structure and size. The application of nanoparticles spans multiple disciplines, including molecular biology, physics, organic and inorganic chemistry, medicine, and materials science. Reducing particle size to the nanoscale leads to unique and enhanced properties in particle size distribution and morphology that are not observed in larger bulk materials. Nanoparticles exhibit both solute and distinct particle phase characteristics. Additionally, the surface-to-volume ratio of nanoparticles is 35-40% higher than that of larger particles or atoms.[1] Composites have gained popularity in various field such as constructional, aeronautical, vehicular, biomedical, industrial etc. Noble metals like gold, silver, and platinum have beneficial health effects and are used to create nanoparticles. Various methods, including physical and chemical reduction as well as photochemical reduction, are commonly employed for the preparation and stabilization of these metallic nanoparticles.

## MATERIALS AND METHODS

### List of materials and their sources

| Sl. No. | Materials | Source |
|---|---|---|
| 1 | Methotrexate | Cipla pvt. Ltd. Mumbai. |
| 2 | Gold chloride | Finar Chemicals, Ahmadabad, India. |
| 3 | Gelatin Powder 240Bloom | SD finechem limited, Mumbai |
| 4 | chitosan | Sigma Aldrich chemicals, Bangalore |
| 5 | Trisodium citrate | SD finechem limited, Mumbai |

### List of instruments and models

| Sl. No. | Equipment's | Company/ Model |
|---|---|---|
| 1 | Digital balance | Essae, Tokyo, Japan |
| 2 | FT-IR spectrometer | Bruker Alpha series, Germany |
| 3 | Spray Dryer | Techno search, Bangalore |
| 4 | Incubator Shaker | Scientek Services, Intron electronics pvt. Ltd |
| 5 | Magnetic stirrer | Remi Equipment's, Bangalore |
| 6 | Digital Ultrasonic Cleaner | Equitron |
| 7 | UV-Visible spectrophotometer | ShimandzuUV-1800 |
| 8 | Super Speed Vacuum Centrifuge | Vision Scientific CO., LTD, Govt of India |

### Preparation of gold nanoparticles:

Chloroauric acid solution of 0.5mM was prepared by dissolving specified amount of chloroauric acid ($HAuCl_4$) in distilled water. This solution was heated to 90°C on a magnetic stirrer. Solution of 1% trisodium citrate solution was prepared by dissolving 100mg in 10ml of water, specific amount of this solution was added to boiling solution of chloroauric acid. Stirring continued at the same temperature until red colored solution was obtained indicating reduction of gold chloride[1]. The gold nanoparticles were purified by dialysis and were purified by dialysis method or by a suitable method.[2]

- The amount of reducing agent added effect the size of nanoparticles formed. Excess of reducing agent leads to aggregation of particles leading to purple color solution.[1]

- If the solution was stirred for more than 40 min after the addition of reducing agent, itleads to formation of larger particles of gold atoms due to disproportion of gold ions.[1]
- If reducing agent was added at 70$^0$C it will lead to the formation of large sized particles.[2]
- The resultant solution was found to be stable for 1week in contrast to this nanoparticle in chitosan and gelatin solution was stable for 2 to 3 days.

**Table 1:** Formulation for preparation of gold Nanoparticle solution:

| Sl. No | Ingredients | F1 | F2 | F3 | F4 | F5 |
|---|---|---|---|---|---|---|
| **1** | Chloroauricacid | 6mg | 5mg | 3mg | 3mg | 12mg |
| **2** | Trisodiumcitrate | 5ml (1.5%) | 3ml(1%) | 2ml(1%) | 1ml(1%) | 4ml(1%) |
| **3** | Distilledwater | 20ml | 20ml | 20ml | 20ml | 20ml |

**Preparation of microspheres**

The prepared nanocomposite was subjected to spray drier.

- This solution was used in the spray drier to obtain microspheres. Inlet temperature andoutlet temperature were set to 140$^0$C and 70$^0$C, VFD 32 and atomization pressure of 1.8 – 2 with a feed rate 4ml /min was obtained while operating the instrument.

***In vitro* drug release**

Different samples of microsphere weighed equivalent to 20mg of methotrexate were suspended in 10ml of phosphate buffer in 10 different number colored bottles of capacity 25ml. These bottles are kept in shaker incubator with a maintained temperature of 37±0.5C$^0$. After 1hr interval time bottle was taken out and subjected to centrifugation [Vision scientific co., Ltd., govt of Karnataka] at a rpm of 10,000 for 30 min and the clear supernant solution was diluted with buffer [7.4pH] which was analyzed under UV-Visible spectroscopy at 240nm.

## RESULTS AND DISCUSSION

A successful attempt was made to formulate microspheres of Methotrexate using polymers. Effect of polymers applied on formulations was assessed**.** The formulated microspheres were characterized for various physicochemical parameters.

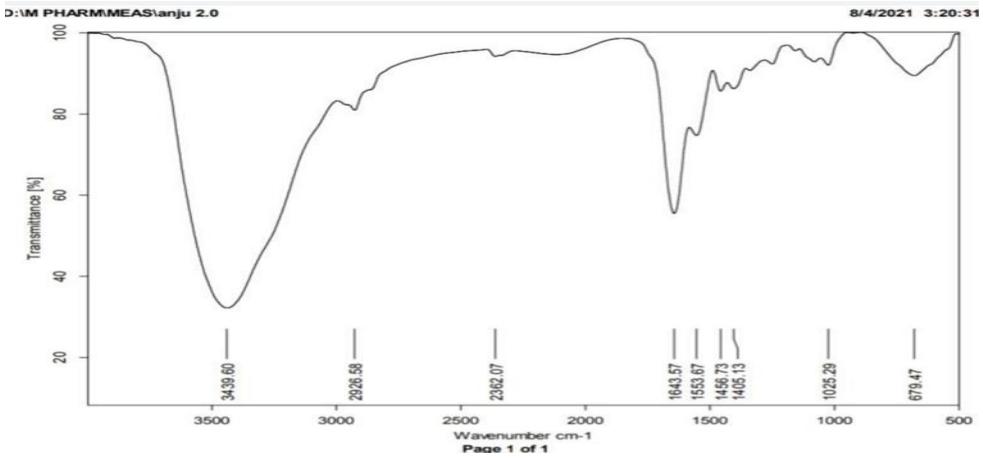


**Figure 1:** IR Spectra of mixture (drug+ polymer)

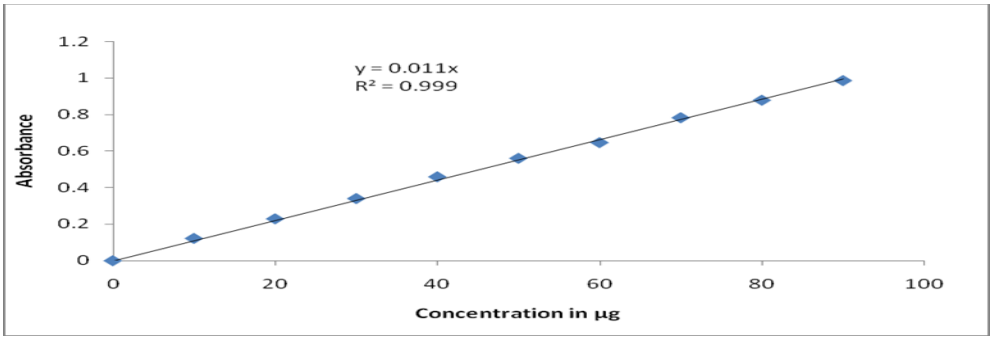


**Figure 2:** Graph of Std. Calibration Curve of Methotrexate in pH7.4 phosphate buffer

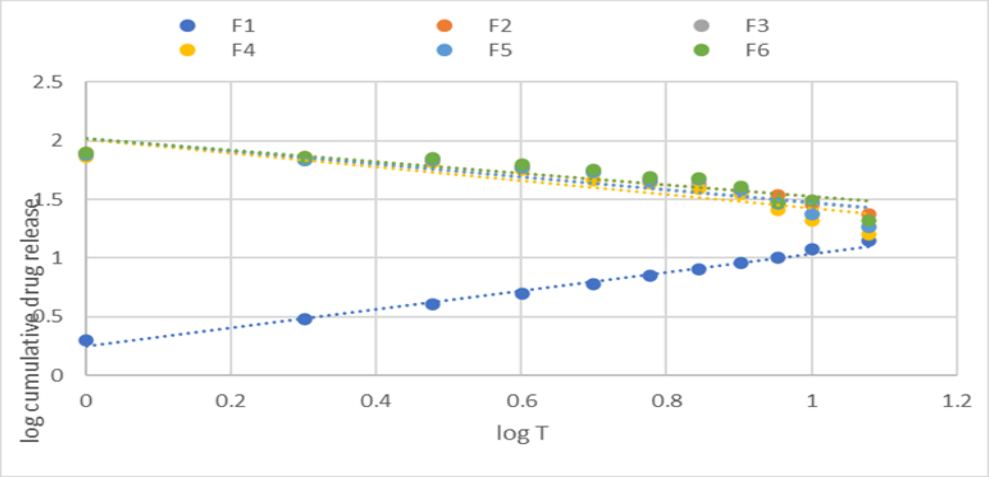


**Figure 3:** Plots of log cumulative% drug released vs log time for different formulations (*in vitro* release studies) [Peppa's]

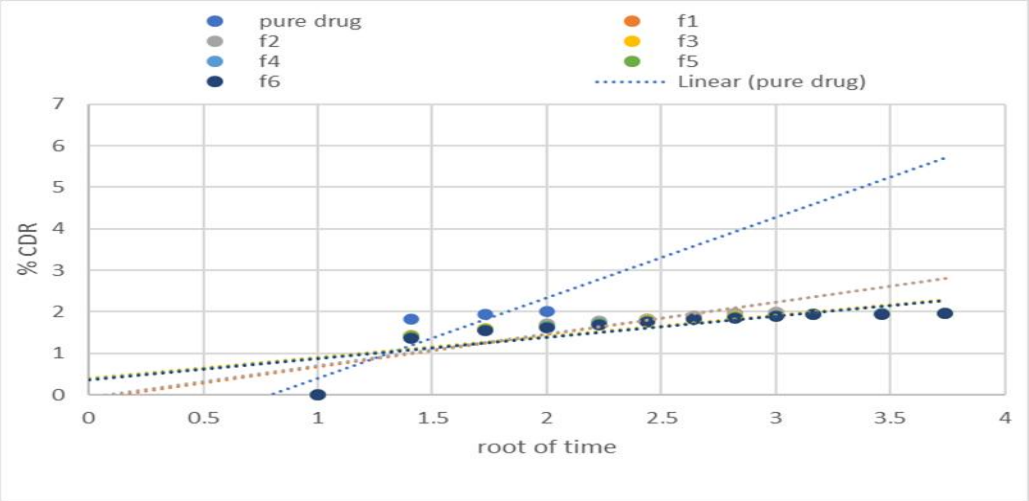


**Figure 4:** Plots of cumulative %drug released vs. root time for different formulations(*in vitro* drug release studies) [Higuchi matrix]

The regression coefficient for formulation F1 to F6 of Peppa's plot was found to be 0.9992, 0.9984,0.9988,0.9966,0.9990,0.9998 respectively which is maintained in figure 3. The regression coefficient for formulation F4 of Higuchi Matrix model is 0.9934,0.9969,0.9966,0.9858,0.5499 respectively. Based on the highest regression values (r) the best fit model for F1, F2, F3, F5, F6, was Peppa's and for F4 was Higuchi matrix which is mentioned in figure 4. Further the 'n' values for the F1, F2, F3, F5, F6. This indicates the release mechanismfollows non-Fickian diffusion.

It was observed that F4 followed Higuchi matrix suggesting drug release by diffusion.

The Peppa's model is widely used when the release mechanism is not well known or when more than one type of release phenomenon could be involved. The 'n' value could be used to characterize different release mechanisms. Peppar-Korsmeyer equation is given as –

%R =Ktn

or log% R= logK+ n logt

where, R=drug release, K=constant, n=slope, t =time

The mechanism of drug release can be predicted by 'n' value. If 'n' value is 0.5 the release pattern would be Higuchi matrix. If 'n' value is less or more than 0.5 and less than 1 the release would be non-Fickian diffusion. If 'n' value is equal to 1 the drug release would be case II transport (Zero release).

If 'n' value is more than 1 the release would be super case II transport. So, the 'n' values for F1, F2, F3, F5, F6, were found to be more than 0.5 and less than 1. Thisindicates that the release follows non-Fickian diffusion matrix. F4 followed Higuchi matrix model.

## CONCLUSION

In the present study a satisfactory attempt was made to develop microspheres of gold nanoparticles for controlled delivery of Methotrexate in the treatment of Arthritis. From the experimental results it can be concluded that:

- Nanoparticles were prepared by dissolving chloroauric acid in distilled waterwhich was heated up to $90^0$C for 40min specific amount of tri sodium citrate was Gold added to chloroauric acid solution.
- Biodegradable natural polymer chitosan is suitable polymer for the preparation of nanocomposite.
- The prepared nanocomposite was subjected to spray drying to obtain microspheres of nanocomposites.
- Formulation F6 showed maximum drug entrapment efficiency of 68.174.
- Particle size analysis revealed that microspheres when incorporated with chitosan and there was an increase in the particle size within the range of 10 m to 30 m with ideal surface morphology for intra articular administration.
- If the solution was stirred for more than 40min after addition of reducing agent, it leads to the formation of larger particles of gold atoms due to disproportion of gold ions.
- Formulation F2 showed maximum cumulative percent drug release.
- Overall, the curve fitting into various mathematical models was found to be average and the in-vitro release of formulations best fitted into the Higuchi's on the basis of drug content, particle size morphology and *in vitro* release kinetics, formulation F4.
- From the results of the present study, it can be concluded that gold nanocomposite prepared by spray drying method with chitosan suitable for thecontrolled release of Methotrexate.
- Methotrexate is a drug of choice in Arthritis, blood/ hormone/ immune system disorders, allergic reactions, certain skin and eye conditions, breathing problems, certain bowel disorders, and certain cancers. Unfortunately, the half-life of Methotrexate is 3to 5 hours. Biodegradable microspheres show a good sustained release of Methotrexate to improve the therapeutic efficacy.
- Gold nanoparticles are non-toxic. Hence in the present study gold nanocomposite of Methotrexate were developed by spray drying method. Six formulations F1 to F6 were prepared by using different amounts of chitosan and gelatin.
- Preformulation studies revealed that the drug Methotrexate and the polymer chitosan were satisfactorily compatible without any change in the chemical nature of the drug.
- The formulations were subjected to various evaluation parameters like % practical yield, entrapment efficiency, particle size distribution, and *in vitro* release studies. The results of all the parameters are tabulated and depicted graphically in the result and discussion section.
- Percentage yield was found to be maximum in formulation F 4. *In vitro* release study was analyzed using various mathematical models. Cumulative percentage drug release with respect to time was found to be highest for formulation F3 and lowest for formulation F6. Based on the regression coefficient values, the best fit model for F1, F2, F3, F5, F6, was Peppa's and F4 was Higuchi matrix.
- The 'n' values of Peppa's suggest non-Fickian release.

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