TIPS

Instability Kinetic Evaluation of Methotrexate in Physiologic pH and Temperature Condition

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Abstract

Today, it's become established that methotrexate (MTX) has good efficacy for the treatment of various disease such as leukemia or rheumatoid arthritis and so on. Although high-dose MTX is easily prescribed for most patients, it can cause severe toxicity including nephrotoxicity. Clearance of the drug from the body following this complication can be delayed. Therefore, it is more desirable to use controlled drug delivery systems for dose reduction and systemic toxicity and drug delivery for a long time. In addition to the above, the chemical instability of the active molecule can lead to a significant reduction in the amount of drug in physiologic conditions. Therefore, it is important to know the nature of the methotrexate reaction at physiological pH and temperature. Considering this, we use phosphate-buffered saline with physiologic pH for release of MTX. As our study showed, methotrexate molecules present instability during the physiological conditions for 48 hours. The present study helps in considering studies of methotrexate taking into account the critical factor of instability, considering the calculated rate constant.

Keywords: Instability, Methotrexate, Physiologic conditions.

1. Introduction

The use of methotrexate (MTX) in the treatment of various diseases, such as leukemia, lymphoma, rheumatoid arthritis, is established. It is a folate anti-metabolite that prevents the synthesis of DNA, RNA, and thymidylates (1). Today, many treatment protocols for malignant diseases, such as acute leukemia, central nervous system (CNS) prophylaxis, use high-dose methotrexate combined with leucovorin (2, 3).

Although high dose MTX is prescribed safely for most patients, it can cause severe toxicity, including nephrotoxicity, that due to the crystallization of methotrexate in a tubular renal lumen, it leads to tubular toxicity (3). This complication can delay the clearance of the drug. Thereby, the drug is prescribed by intravenous injection, which is very painful and unacceptable to the patient. Hence, the use of controlled drug delivery systems is more desirable to reduce the dose and systemic toxicity and delivery of the drug ultimately.

Many different polymeric compounds are nowadays used in new drug delivery systems to solve MTX treatment problems, such as polymeric nanoparticles (4), microspheres (5), solid lipid nanoparticles (6), human serum albumin, liposomes, in situ forming hydrogels, polymeric micelles, dendrimers, carrier erythrocyte, and nanotechnology-based vehicles such as hydrogel nanoparticles (7-9), carbon nanotubes, magnetic nanoparticles, and gold nanoparticles (10, 11). Some other drug delivery systems have been modified with targeting ligands for active targeting purposes target.

In addition, the chemical instability of the

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active molecule results in a significant reduction in the amount of the therapeutic agent in the dosage. pH is one of the most critical factors affecting the stability of the drug, which in turn is considered as an essential criterion in determining the appropriateness of the drug for that specific therapeutic approach as mentioned above (12). In the alkaline pH range, N10-methyl folic acid is the product of MTX degradation by alkaline hydrolysis (13). Hence, it usually develops a pH-rate profile to measure pH, in which the drugs are more susceptible to degradation, and then formulate the drug in a formula with different pH using a buffer to reduce its degradation (14). It is important to know the nature of the reaction of methotrexate in physiological pH. This kinetic study reports the reaction of methotrexate in physiological conditions at 37 °C. Considering this, we use phosphate-buffered saline with physiologic pH for release of MTX.

2. Material and methods

2.1. Materials

Methotrexate was kindly supplied by Loghman Pharmaceutical Co. (Tehran, Iran). All other chemicals, solvents, and reagents used were of chemical or analytical grade, as needed, and were purchased locally.

2.2. Drug assay

The reversed-phase high-performance liquid chromatography (HPLC) method was used to determine the concentration of MTX in samples. The chromatography system consisted of a C18 column (Eurosphere 100-5 C18, 150×4.6 mm, Germany) as a stationary phase and a mixture of phosphate buffer (0.01 M, pH 3.9) and acetonitrile (85:15) as the mobile phase. An analyte was detected at a wavelength of 307 nm by a UV detector (Knauer, model k-2600, Berlin, Germany). Chromatograms were analyzed using compatible software (EZChrom Elite®, Germany) (15, 16).

2.3. Stability study

Stability kinetic of MTX was investigated while suspended in physiologically simulated medium and temperature (i.e., the temperature of 37 °C and phosphate-buffered saline, (PBS; pH of 7.4, (17)). A new assembly was designed for these studies. Briefly, our system consisted of two Franz cells as the donor and receptor phase containers separated by a dialysis membrane (Dialysis Tubing Cellulose Membrane, D9527-100FT, size: 43 mm×27 mm, Sigma-Aldrich, USA) with a predetermined surface area. The vessels were double-jacketed with 37 °C water circulating between the jacket walls throughout the study (Figure 1). In each experiment, a free drug solution (20 µg in 23 ml PBS; pH of 7.4), was used as the donor phase. As the receptor phase, the same volume of PBS was added to the receptor compartment, and aliquots (about 100 µl) of the release medium (i.e., receptor phase) were withdrawn at predetermined time points (i.e. 0, 0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 12, 24, 28, 30, and 48 h). The amount of MTX in the release medium was performed using the mentioned HPLC method. After each measurement, the same fresh PBS volume was performed in the receptacle. All experiments were repeated three times.
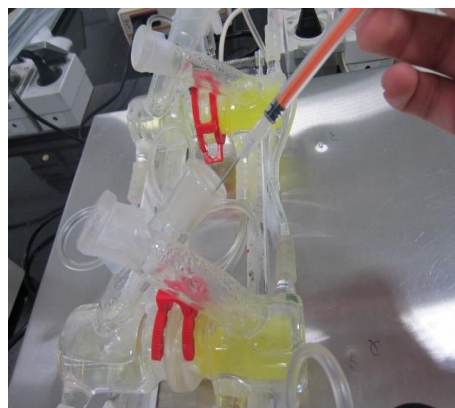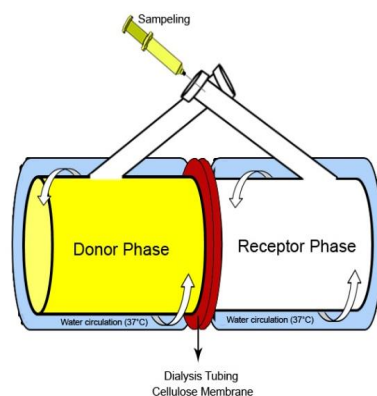

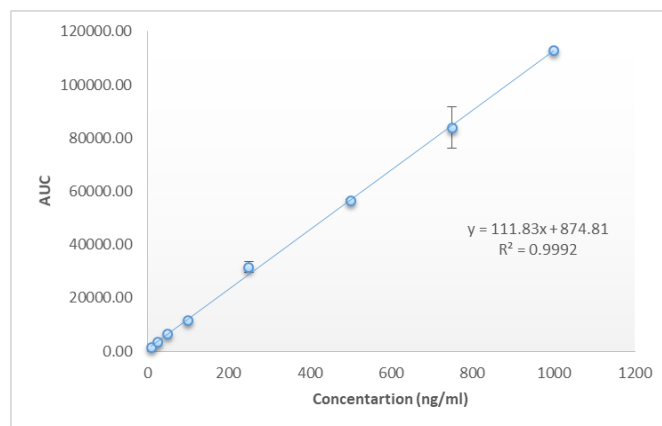Figure 1. our new assembly system for *in vitro* release study.

Figure 2. Calibration curve of methotrexate in PBS phase (n=3).

2.4. Kinetic study

A first-order condition for the kinetic study of methotrexate in a physiological pH range at 37 °C was calculated using a stability indicating HPLC assay.

First-order equation

$$\ln(1-F) = -k1t \qquad (Eq.1)$$

Where "k1" stands for first-order release rate constant (18).

3. Results and discussion

3.1. Drug assay

The method produced linear relationship throughout the methotrexate concentration range of 10-1000 ng/ml in PBS phase. The calibration curve is shown in Figure 2.

3.2. Kinetic study

The stability profile of MTX and its related kinetic study is shown in Figure 3.

As seen in Figure 3, the methotrexate con-

centration in physiologic conditions is reduced during the time. It could be due to the instability of these drug molecules. First-order kinetic showed the linear relationship between the logarithmic scales of the drug remained percent vs. the time. In this curve, fitting the first constant of drug instability is calculated from the slope of the equation (i.e., 0.008). It is very critical during the studies on methotrexate.

4. Conclusion

In many studies of drug delivery and formulation design, researchers examine various parameters, such as the profile of drug release in physiological conditions. The analysis of information obtained from these studies can play an essential role in the *in vitro-in vivo* relationship. Any confounding factor in these studies results in invalidating the results. One of these factors is drug instability, which is very important. Methotrexate is an important therapeutic agent used in many studies. As our study showed, meth-
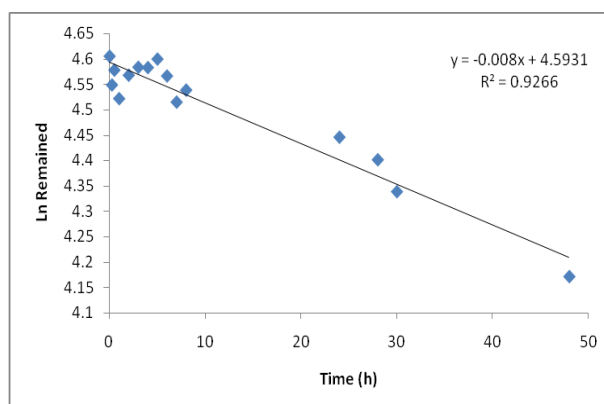

Figure 3. The MTX stability profile at 20 µg/23 ml concentration in release study condition (i.e. buffer solution with pH value of 7.4 at 37 °C.) (n=3).

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Conflict of Interest
None declared.

5. References

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