Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 3174-3179 | ISSN:2251-6727

In vitro Drug Release of Novel Polysaccharide 5-FU conjugate

Haribabu Thiriveedi[1*], Manjunatha P Mudagal[1]

[1*]Assistant Professor, Department of Pharmacology, Acharya & BM Reddy College of Pharmacy, Soldevanahalli, Bengaluru 570106, Karnataka, India.

[1]HOD & Professor, Department of Pharmacology, Acharya & BM Reddy College of Pharmacy, Soldevanahalli, Bengaluru 570106, Karnataka, India.

(Received: 04 February 2024 Revised: 11 March 2024 Accepted: 08 April 2024)

KEYWORDS

5-FU Conjugate, in-vitro diffusion, kinetic models, alkaline hydrolysis, CS-FUAC.

ABSTRACT:

As the second greatest cause of mortality in the US, cancer is a serious global public health issue. Advanced-stage disease and mortality rates may rise as a result of delayed diagnosis and treatment. 5-fluorouracil (5-FU) is a popular chemotherapeutic medication used to treat a range of malignancies, including colorectal, breast, and head and neck cancers. The creation of 5-FU conjugates, in which the medication is chemically bonded to polymers, nanoparticles, or other macromolecular carriers, is one possible strategy. An essential first step in assessing 5-FU's release properties from its conjugate formulation is the in-vitro diffusion research. The results from this study can be used to create release profiles, which can then be examined to learn more about the mechanisms and kinetics of release, including diffusion, swelling, and erosion. The results obtained above drug release follows first order kinetics as witnessed with high R^2 value (0.955). This indicates drug release is concentration dependent. The results of alkaline hydrolysis of CS-FUAC have shown that the 5-FU have good ability to form conjugation with chitosan.

1. Introduction

Cancer is a major public health problem worldwide and is the second leading cause of death in the United States. Delays in diagnosis and treatment may lead to an uptick in advanced-stage disease and mortality. Cancer was first recognized as a growing tissue mass, a tumor[1]. They could observe what it looked like, how fast it grew, and how it often appeared to take a "bite" out of the body. The tumors themselves seemed, at times, to spread to other parts of the body and were recognized as fatal. Two things are evident at this stage of understanding2. First, it aligns with a disease of abnormal and uncontrolled growth with the capacity to spread. Second, there was no distinction made between the concepts of tumor and cancer. Even today, these terms are often used interchangeably[3].

A common chemotherapeutic drug used to treat a variety of cancers, such as colorectal, breast, and head and neck cancers, is 5-fluorouracil (5-FU). Despite its efficacy, 5-FU's short half-life, systemic toxicity, and quick metabolism frequently prevent it from being used clinically. Different drug delivery methods and conjugation techniques have been developed to get around these restrictions, improving the therapeutic efficacy and lowering the side effects of 5-FU[4].

The creation of 5-FU conjugates, in which the medication is chemically bonded to polymers, nanoparticles, or other macromolecular carriers, is one possible strategy. These conjugates seek to enhance 5-FU's pharmacokinetic profile, enable targeted administration to tumor locations, and offer the medication in a controlled release format. In order to minimize peak plasma levels that may be harmful while preserving therapeutic concentrations that are effective for a prolonged amount of time, the controlled release of 5-FU from its conjugate is essential[5, 6].

An essential first step in assessing 5-FU's release properties from its conjugate formulation is the in-vitro diffusion research. Under carefully monitored laboratory settings, this kind of research mimics the

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 3174-3179 | ISSN:2251-6727

drug release process and offers crucial insights into the release kinetics, mechanism, and possible effectiveness of the drug delivery system. Researchers can forecast the conjugate's in-vivo performance and optimize the formulation parameters by using in-vitro diffusion models[7, 8.]

The results from this study can be used to create release profiles, which can then be examined to learn more about the mechanisms and kinetics of release, including diffusion, swelling, and erosion. Researchers can learn more about the physiological behavior of the 5-FU conjugate and how changes to the conjugate formulation may affect its release properties by fitting the data to different kinetic models[9].

In conclusion, the in-vitro diffusion research of a 5-FU conjugate is an important experimental strategy that offers insightful knowledge about the drugs controlled release characteristics. This is a crucial phase in the creation and refinement of cutting-edge drug delivery systems intended to maximize 5-FU's therapeutic potential and reduce its side effects.

2. Methodology

In-vitro Diffusion Study of 5 FU Conjugate:

In-vitro diffusion studies for 5 FU conjugate were carried out by using dialysis bag technique. In this method the 5 FU conjugate was placed in dialysis bag which then immersed in beaker containing 100 ml phosphate buffer saline (pH-7.4). Temperature was stabilized at 37°C ± 0.5. and kept under continuous stirring. The drug which diffuses from 5FU conjugate in phosphate buffer saline was periodically pipetted out and the same amount was replaced with fresh phosphate buffer saline. Absorbance was analyzed by UV spectrophotometer using Agilent Technologies Cary 60 UV-Vis to calculate the cumulative drug release profile of drug[10,11,12.]

Kinetic Modelling of *In-vitro* Drug Diffusion Profile:

Data obtained from the in-vitro diffusion study of drug was fitted to various kinetic models. The models were fitted to zero order, first order, Higuchi and Korsmeyer-Pepas to ascertain the kinetic modeling of the drug release. The (R2) regression co efficient was adopted to obtain the most appropriate model[13,14,15].

i. Zero order Kinetics: The zero order models describe the systems where the drug release rate is independent of its concentration. The plot was % cumulative drug released vs. time.

ii. First order kinetics: The first order model describes the release from the systems where release rate is concentration dependent. The plot was % log drug remaining vs. time.

iii. Higuchi model: Higuchi model was developed on the basis of Fick's law and it describes the fraction of drug release from a matrix is proportional to square root of time. The plot was % drug released vs. square root of time.

iv. Korsmeyer-Peppas model: It describes the drug release from the polymeric system in which release deviates from Fickian diffusion.

Table 1: Release Pattern based on N values

N Value	Release Pattern
<0.5	Fickian diffusion
0.5<n<1	Non Fickian
0.8-1	Anomalous diffusion
1	Zero order, case-II transport
>1	Super case- II transport

3. Results

In-vitro drug diffusion study:

The %CDR data for 5 FU Conjugate are given in Table 2.

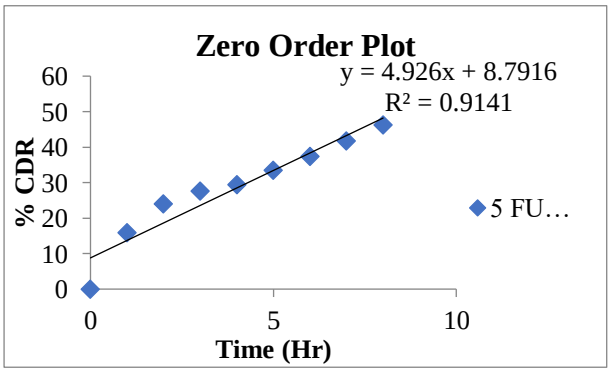

Figure 1: Zero order plot for 5 FU conjugate.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(3), 3174-3179 | ISSN:2251-6727

Table 2: %CDR data for 5 FU Conjugate

S.No	time (h)	sq rt time	log time	%CDR	Drug rmng	log% drug rmng	Mt/Minf	log Mt/Minf
1	0	0.00	#NUM!	0.000	100.000	2.0000	0.0000	#NUM!
2	1	1.00	0.000	15.920	84.080	1.9247	0.3439	-0.464
3	2	1.41	0.301	24.130	75.870	1.8801	0.5213	-0.283
4	3	1.73	0.477	27.730	72.270	1.8590	0.5990	-0.223
5	4	2.00	0.602	29.510	70.490	1.8481	0.6375	-0.196
6	5	2.24	0.699	33.480	66.520	1.8230	0.7233	-0.141
7	6	2.45	0.778	37.530	62.470	1.7957	0.8108	-0.091
8	7	2.65	0.845	41.870	58.130	1.7644	0.9045	-0.044
9	8	2.83	0.903	46.290	53.710	1.7301	1.0000	0.000

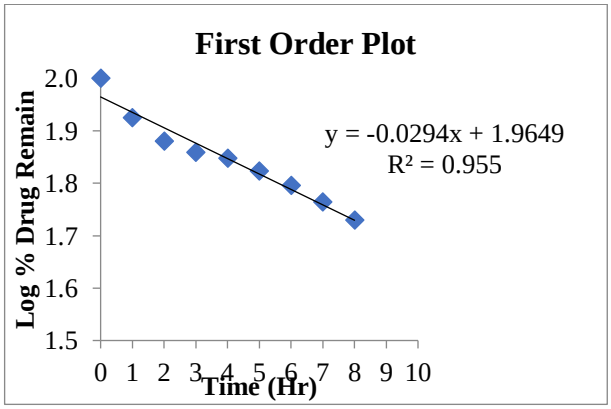

Figure 2: First order plot of 5 FU conjugate.

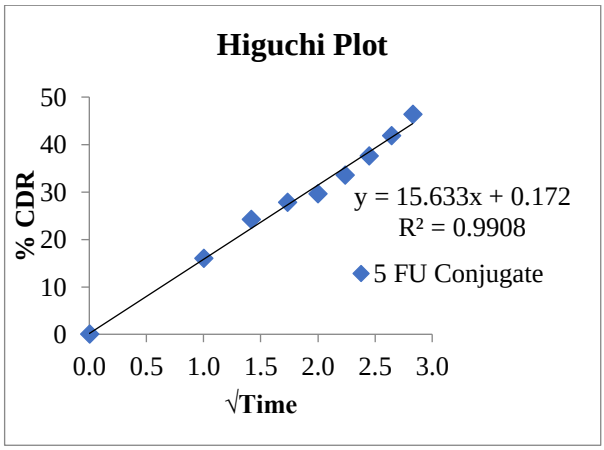

Figure 3: Higuchi plot of 5 FU conjugate.

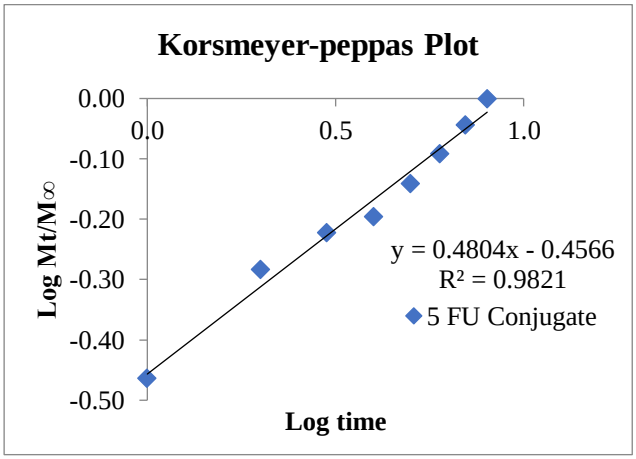

Figure 4: Korsmeyer-peppas plot for 5fu conjugate.

Table 3: Release Kinetic Data of 5 FU conjugate.

	Zero Order	First Order	Higuchi	Korsmeyer-Peppas	Korsmeyer-Peppas
5 FU Conjugate	R^2	R^2	R^2	R^2	n value
	0.9141	0.955	0.9908	0.9821	0.48

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 3174-3179 | ISSN:2251-6727

The Percentage of Drug Content in the Conjugate:

The percentage of FUAC in the conjugates was determined by subjecting the prodrug to treatment in basic media to hydrolyze the amide bond, followed by absorbency measurements at 273 nm[16].

Alkaline hydrolysis of CS-FUAC:

The FUAC content in the conjugates was measured by the basic hydrolysis of the amide bond between the CS and FUAC. The alkaline hydrolysis of CS-FUAC involves reaction with hydroxide ions to yield FUAC. In the alkaline hydrolysis of amides the hydroxide ion nucleophile attacks the carbonyl carbon in a nucleophilic acyl substitution reaction. Upon hydrolysis, an amide converts into a carboxylic acid and an amine or ammonia (which in the presence of acid are immediately converted to ammonium salts). The alkaline hydrolysis of CS-FUAC is depicted in Figure 5.

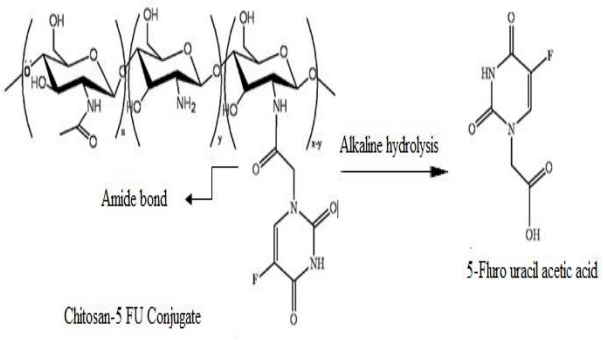

Figure 5: Alkaline hydrolysis of CS-FUAC

A solution of CS-FUAC conjugates was prepared by dissolving the compound in 1 N NaOH (1 mg/mL). 1N NaOH solution for alkaline hydrolysis studies was prepared by dissolving 4 gm of NaOH in 100 ml of distilled water in 100 ml volumetric flask at room temperature (22°C) and the resultant NaOH solutions used for studies.

Calibration Curve

Calibration curve of the drug chitosan conjugate (CS-FUAC) was developed to found out the linearity between concentration of drug in the solution and its absorbance. A stock solution is prepared by dissolving 50 mg of pure 5-FUAC in 50mL of distilled water. Different working standards viz., 10µg/mL, 20µg/mL, 30µg/mL, 40µg/mL and 50µg/mL were prepared by appropriate dilutions. Absorbance of those solutions at the λ max 273 nm is measured. The linear regression analysis was performed. The Beer's -Lambert's law was obeyed in the concentration range between 10 and 50 µg/mL implies a perfect linearity was found.

A solution of CS-FUAC conjugates was prepared by dissolving the compound in 1 N NaOH (1 mg/mL), and their absorbance was also recorded at 273 nm.

$$\%\left(\frac{w}{w}\right)\text{ of FUAC loading} = \frac{\text{FUAC amount}}{\text{CS} - \text{FUAC conjugates amount}} \text{x} 100$$

Table 4: Absorbance of different concentration of 5-FUAC and CS-FUAC at 273 nm

S.No	Concentration (µg/mL)	Absorbance
1.	10	0.0590
2.	20	0.1083
3.	30	0.2823
4.	40	0.4820
5.	50	0.8553
6.	Conjugate (10 µg/mL)	0.0491

The percentage of FUAC in conjugates was measured by comparison with the standard. The following equation was used to calculate the percent of drug loading:

Table 5: Reaction data for CS-FUAC conjugates

Analyte	Concentration (µg/mL)	Absorbance
Standard (FUAC)	10	0.0590
CS-FUAC conjugates	10	0.0491

$$\%\left(\frac{w}{w}\right)\text{ of FUAC loading} = \frac{8.3}{10}\text{ X } 100 = 83\%$$

The percentage of FUAC in conjugates was measured by comparison with the standard. The percentage of FUAC conjugated to CS was found to be 83% when the molar ratio was kept at 1:1. The results of alkaline hydrolysis of CS-FUAC have shown that the 5-FU have good ability to form conjugation with chitosan.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 3174-3179 | ISSN:2251-6727

4. Discussion

The drug release pattern of 5 FU Conjugate was conducted for 8 h using KC-diffusion cell using phosphate buffer saline (pH 7.4) as the diffusion medium. Dialysis membrane-12,500 molecular weight cutoff, previously socked in buffer solution for 24h was used as semi permeable membrane. In this the known amount of 5 FU conjugate(100mg) was placed in dialysis bag which then immersed in beaker containing 100 ml phosphate buffer saline. Temperature was stabilized at 37°C ± 0.5. The drug which diffuses from 5FU conjugate in phosphate buffer saline was periodically pipetted out and the same amount was replaced with fresh phosphate buffer saline and Absorbance was analysed by UV-Visible spectrophotometer at 320nm.

The data obtained from in vitro diffusion studies were evaluated for zero order, first order, Higuchi, and Korsmeyer-peppas models to check the phenomena controlling the drug release. The goodness of fit was evaluated using the correlation coefficient (R^2) values and mechanism of drug release is described based on release exponent, 'n' value.

The drug release profile and kinetics of drug release are necessary to predict the in vivo drug release pattern. The results of cumulative drug release fitted into various mathematical models as shown in **Table 2**. The drug release pattern was found to follow first order kinetics as indicated by their high regression coefficient (R2) value of 0.955. Drug release was found to be highly linear, and close to infinity. Formulation showed highest regression value (R2 = 0.9908) for Higuchi equation, indicating the drug release follows diffusion mechanism (Higuchi T 1963). Further, 'n' value for Korsmeyer-Peppas equation was found to be 0.48 suggesting that release of drug follows fickian diffusion mechanism. Therefore we assume that drug release from this drug-polymer conjugate must be combination of both diffusion and erosion mechanism (Faith A 2010).

5. Conclusion

The results obtained above drug release follows first order kinetics as witnessed with high R2 value (0.955). This indicates drug release is concentration dependent. Further the n value obtained for Korsmeyer-peppas model is found to be 0.48 which indicates fickian diffusion. Results are shown in table 2. The percentage of FUAC conjugated to CS was found to be 83% when the molar ratio was kept at 1:1. The results of alkaline hydrolysis of CS-FUAC have shown that the 5-FU have good ability to form conjugation with chitosan.

ACKNOWLEDGEMENT

The authors express gratitude to the Management and Principal of Acharya & BM Reddy College of Pharmacy, Bengaluru, for providing necessary facilities to carry out this project.

Conflict Of Interest

The authors declare no conflict of interest

Refrences

1. Frankell AM, Dietzen M, Al Bakir M, Lim EL, Karasaki T, Ward S, Veeriah S, Colliver E, Huebner A, Bunkum A, Hill MS. The evolution of lung cancer and impact of subclonal selection in TRACERx. Nature. 2023 Apr 20;616(7957):525-33.
2. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. Ca Cancer J Clin. 2023 Jan 12;73(1):17-48.
3. Brown JS, Amend SR, Austin RH, Gatenby RA, Hammarlund EU, Pienta KJ. Updating the definition of cancer. Molecular Cancer Research. 2023 Nov 1;21(11):1142-7.
4. Nikam AN, Jacob A, Raychaudhuri R, Fernandes G, Pandey A, Rao V, Ahmad SF, Pannala AS, Mutalik S. Topical Micro-Emulsion of 5-Fluorouracil by a Twin Screw Processor-Based Novel Continuous Manufacturing Process for the Treatment of Skin Cancer: Preparation and In Vitro and In Vivo Evaluations. Pharmaceutics. 2023 Aug 22;15(9):2175.
5. Gahtani RM, Alqahtani A, Alqahtani T, Asiri SA, Mohamed JM, Venkatesa Prabhu S, Muluneh EY. [Retracted] 5-Fluorouracil-Loaded PLGA Nanoparticles: Formulation, Physicochemical Characterisation, and In VitroAnti-Cancer Activity. Bioinorganic Chemistry and Applications. 2023;2023(1):2334675.
6. Ali A, Madni A, Shah H, Jamshaid T, Jan N, Khan S, Khan MM, Mahmood MA. Solid lipid-based nanoparticulate system for sustained release and enhanced in-vitro cytotoxic effect of 5-fluorouracil

on skin Melanoma and squamous cell carcinoma. Plos one. 2023 Feb 28;18(2):e0281004.

7. Yadav BK, Patel G. Fabrication and characterization of 5-FLUOROURACIL loaded multilayered electrospun nanofiber for the treatment OF SKIN cancer. Journal of Drug Delivery Science and Technology. 2023 Sep 1;86:104582.
8. He J, Duan Q, Ran C, Fu T, Liu Y, Tan W. Recent progress of aptamer–drug conjugates in cancer therapy. Acta Pharmaceutica Sinica B. 2023 Apr 1;13(4):1358-70.
9. Sarkar S, Kiren S, Gmeiner WH. Review of Prodrug and Nanodelivery Strategies to Improve the Treatment of Colorectal Cancer with Fluoropyrimidine Drugs. Pharmaceutics. 2024 May 29;16(6):734.
10. Yan GP, Zong RF, Li L, Fu T, Liu F, Yu XH. Anticancer drug-loaded nanospheres based on biodegradable amphiphilic *ε*-caprolactone and carbonate copolymers. Pharma Res 2010;27(12):2743-52.
11. Chidambaram N, Burgess DJ. A novel in-vitro release method for submicron sized dispersed systems. AAPS Pharm Sci 1999;1(3):32-40.
12. Kumaresan C, Satishkumar K. Development of an inhaled sustained release dry powder formulation of salbutamol sulphate, an antiasthmatic drug. Indian J Pharm Sci 2016;78(1):136-42.
13. Arifin DY, Lee LY, Wang CH. Mathematical modeling and simulation of drug release from microspheres: Implications to drug delivery systems. Adv Drug Deliv Rev 2006;58(12-13):1274-1325.
14. Dash S, Murthy PN, Nath L, Chowdhury P. Kinetic modeling on drug release from controlled drug delivery systems. Acta Pol Pharm. 2010;67(3):217-23.
15. Gautam S, Mahaveer S. Review: in-vitro drug release characterization models. Int J Pharma Res 2011;2(1):77-84.
16. Mohammed MO, Hussain KS, Haj NQ, Preparation and Bioactivity Assessment of Chitosan-1-Acetic Acid-5-Flurouracil Conjugates as Cancer Prodrugs. Molecules. 2017 Nov 8;22(11).