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MONITORING THE EFFICIENCY OF 5- FLUOROURACIL RELEASED FROM SYNTHESIZED COMPOSITE OF POLY B- AMINO ESTER AS ANTITUMOR AND ANTIBACTERIAL

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ABSTRACT

Controlled release of poly β- amino ester loaded with 5- fluorouracil as drug was synthesized through Michael addition polymerization under specific conditions. The 5- fluorouracil was loaded on the prepared polymeric material near the end of reaction. The investigated composite was subjected to release 5- fluorouracil (active agent) in distilled water at room temperature for different time periods. The sustained release of the active agent was measured spectrophotometrically. The cytotoxicity of released 5- fluorouracil was monitoring as an anti-proliferative agent against human liver cancer cell line (HEPG2) as well as antimicrobial against both Gram positive and Gram negative bacteria. The sustained release of the active agent results showed that, poly β- amino ester polymer will be a promising carrier for the antitumour drugs. Results of the *in vitro* cytotoxic activity of the released 5-fluorouracil showed a sustained antiprolefrative activity against the tested human liver cancer cell line for 26 days. Moreover, results of the antimicrobial study revealed that the most potent inhibitory activity was against Enterotoxigenic *S. aureus* ATCC 13565 which show hindrance zone of about 48 mm against the tested ref strain.

KEYWORDS: 5-fluorouracil, poly β- amino ester, drug delivery, *invitro*- release, anticancer activity, antimicrobial activity.

INTRODUCTION

The severity of side- effects associated with conventional cancer chemotherapy has promoted the development of a variety of drug delivery approaches. One promising approach for targetable drug delivery is the use of polymers, and several polymer- drug conjugates are currently in advanced clinical trials via controlled release system. Controlled release technique is an attainable and desirable characteristics for drug delivery system.[1-3] This technique is a good mean for providing rate controlled release of active materials to overcome many of the short comings of traditional or conventional drug delivery routes.[4,5] The conventional drug delivery gives sharp increases of drug concentration at potentially toxic levels followed by relatively short period of the therapeutic level and the drug concentration drops off until re- administration. The desired drug release was provided by controlling biodegradable polymers contained drug.[6] The release of drug depends on different aspects as the type of polymer and the response to the environment as occurs in smart systems such as thermo responsive and pH sensitivity.

Polyβ-amino ester is a cationic polymer easily synthesized by addition polymerization reaction under certain specific condition[7] and can be used as carrier for drug therapeutic applications.[8]

5- fluorouracil is a drug that is a pyrimidine analog which is used in the treatment of cancer. Over the past 30 years, 5-fluorouracil has been used for solid tumours, including advanced breast cancer and adenocarcinomas of gastrointestinal tract.[9-11] However, despite its beneficial effects, administration of 5-fluorouracil is accompanied by disorders of the bone marrow and epithelium of gastrointestinal tract, which seriously limit its therapeutic effect.[12,13] Moreover, 5-fluorouracil has very short half –life in plasma as a result of its fast metabolism in liver, and a sustained intravenous infusions often required to maintain an adequate drug concentration in the blood.

This work aims to construct the controlled release system from the synthesized poly β- amino ester loaded with 5-fluorouracil to apply an overview on the release rate of 5-fluorouracil and evaluating this system as promising technique for anti-tumor activity against liver cancer cell lines and anti-bacteria as well.

MATERIALS AND METHODS

Materials

Piperazine and 1, 4-butanediol diacrylate were purchased from Sigma-Aldrich. Tetrahydrofuran (THF) and n-hexane were obtained from Aldrich. 5- fluorouracil as anticancer bioactive drug was purchased fom Merck.

Methods

Synthesis of Poly(*β*-Amino Ester)

The polymer synthesis was carried out according to Michael addition polymerization reaction from 1,4-butandiol diacrylate and piperazine.[7]

Drug encapsulated polymeric capsules

The poly β- amino ester (1.5g) was mixed with (10 mg) of 5- fluorouracil as antitumor near the end of reaction preparation, then divided into discs. The drug polymer discs were left to equilibrate for 24 hours at room temperature.

Surface Characterization and analysis

Scanning electron microscopy was performed on gold coated samples using a Polaron sputter coater. A JXA-840A Electron probe micro- analyzer (JEOL, Japan), operating typically at 30 KV, was employed for morphology measurement and evaluation of the samples.

Measurement of the release rate of 5-Fluorouracil from polymeric discs

The polymeric drug-loaded capsules prepared was immersed in 100 mL distilled water at room temperature. 5mL of the motherlquor was removed and then diluted with 5mL of fresh distilled water at different intervals of time. The absorbance intensity of 5- fluorouracil released was next measured at 222 nm using a UV–Vis spectrophotometer. The standard curve with a linear range of 10−100 μg/mL was plotted, as shown in Figure 1.

Anticancer testing

Measurement of potential cytotoxicity was done using SRB assay.[8] The timely released drug (5-fluorouracil) was subjected to a screening system for evaluation of its antitumor activity against HEPG2 liver cancer.

Antimicrobial testing

Antibacterial activity of the time intervals released drug (5- fluorouracil) was *in vitro* evaluated using agar well diffusion test[14,15] against the following tested stains; Gram positive bacterial reference stains; *Strept. mutans* ATCC 25175, *Cl. perfringens* ATCC 13124 and Enterotoxigenic *S.aureus* ATCC 13565. As control positive Antibiotic (AMC30) (30µg/ml) was used as standard antibacterial, while dimethyl sulphoxide (DMSO) was used as control negative. The test was repeated in triplicate and the mean zone of inhibition was tabulated.

RESULTS AND DISCUSSION

Controlled release of 5- fluorouracil from Poly β- amino ester

The controlled release of poly β-amino ester containing 5-fluorouracil was prepared from 1,4-butanedioldiacrylate and piprazin as described in previous work.[7] The prepared polymeric material was shaped into discs containing the drug. The discs were subjected to release 5-fluorouracil in distilled water at 25^0C. The release amount was estimated spectrophotometrically. The release rate was represented in figure (2). The release was obtained through diffusion dissolution mechanism. Upon exposure to water, the water penetrates inside the disc, then take amount of the drug (5-fluorouracil) to outside. As the surface layer of the disc depleted the tortous pass and pore like structure were formed, the diffusion of water is going into the disc to solve some of drug (dissolution) and coming out. This process was repeated and continued through period of exposure and consequently sustained release of drug was obtained. The rate of drug loss varies according to increasing the growing tortous passes and the pore.

From figure (2), the sustained release was established to about 26 day. It was found that the release rate at the beginning was slow and increased linearly as the time of exposure was increased for the first 12hours. After 24 hours, the steady state was accomplished and release became near constant to be 70and 60 µg/ml/day. This indicated that poly β-amino ester was a good carrier for 5-fluorouracil achieving significant slow release technique. This helps to maintain the effective concentration of 5- fluorouracil for long periods of time.

Characterization and evaluation of surface texture of the investigated polymeric composites

The characterization of surface texture of the investigated slow release composites were carried out using scanning electron microscope (SEM). Figure 3(a,b,c), illustrates the

micrographs of the prepared polymeric composites before and after loading 5-fluorouracil drug and after releasing the drug in distilled water.

It was found that, Figure (3a) shows good homogenous and compatible surface of prepared polymer (without drug). Complete dispersion of the drug through the network structure of poly β-amino ester (Figure 3b).

Figure (3c) shows some tortuous pass and pore like structure formed after releasing the drug according the diffusion dissolution mechanism.

In vitro cytotoxicity of the released drug from the polymer

Table 1 shows the efficiency of the drug released from the prepared polymer on growth inhibition and survival of HEPG2 liver cell line. The cytotoxicity is dependent on the time of exposure, as the longer the time of sustained release of the drug, the higher growth inhibition activity.

Antimicrobial activity:

Antimicrobial activity of the released drug (5-fluorouracil) was evaluated against Gram Positive bacterial reference stains; *Strept. mutans* ATCC 25175, *Cl. perfringens* ATCC 13124 and Enterotoxigenic *S.aureus* ATCC 13565. As control positive Antibiotic (AMC_{30}) (30µg/ml) while dimethyl sulphoxide (DMSO) was used as negative control. Results revealed that the released 5-fluorouracil after 7d, 22d and 26d gives the highest antibacterial activity against Gram positive bacterial reference strains; Enterotoxigenic *S.aureus* ATCC 13565 strain with a mean zone of inhibition equal 48 mm, followed by the released compounds at 5d, 11d and 14d (46mm) then 9d (44mm). On the contrary, these compounds have very weak antibacterial activities against *Cl.perferingens* ATCC 13124 and have no inhibitory effect against *Strept. mutans* ATCC 25175, as shown in Table (2) and figure (4).

Table1: Anticancer potency of 5- fluorouracil released from Poly β- Amino ester polymer against HEPG2 cell- line.

Conc (µg/ml)	Exposure time, day	SURVIVING%	INHIBITION%
10	0.042	86.44444	13.555556
20	0.125	81.71486	18.28514

30	0.25	67.46988	32.53012
60	0.5	66.66667	33.33333
70	1	63.85542	36.14458
70	2	64.88889	35.11111
70	3	61.1245	38.8755
70	5	62.73092	37.26908
70	7	66.30522	33.69478
70	9	61.5261	38.4739
60	11	64.33735	35.66265
60	14	65.14056	34.85944
60	18	70.36145	30.638554
60	22	66.38554	33.614458
60	26	60.24096	39.75904

Table 2: Antimicrobial activity of 5- fluorouracil released from Poly β- Amino ester

Sample no	**Release rate of 5- fluorouracil Conc (µg/ml)**	***Zone diameter,mm for***		
		***Strept. mutans* ATCC 25175**	***Cl.perferingens* ATCC 13124**	***S.aureus* Enterotoxigenic ATCC 13565**
1h	10	-ve	-ve	17
3h	20	-ve	-ve	28
6h	30	-ve	-ve	30
12h	60	-ve	-ve	38
24h	70	-ve	12s	38
48h	70	-ve	16s	38
5d	70	-ve	-ve	46
7d	70	-ve	-ve	48
9d	70	-ve	-ve	44
11d	60	-ve	-ve	46
14d	60	-ve	-ve	46
22d	60	-ve	-ve	48
DMSO		-VE	-VE	-VE
ANTIBIOTIC (AMC_{30}) /ANTIMYCOTIC (AMB_{30})		15	13	30

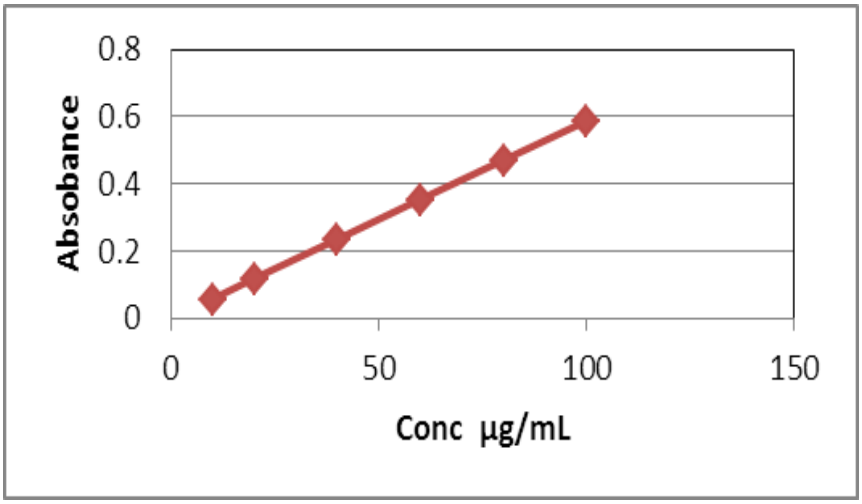

Fig. 1: Standard curve of 5- fluorouracil

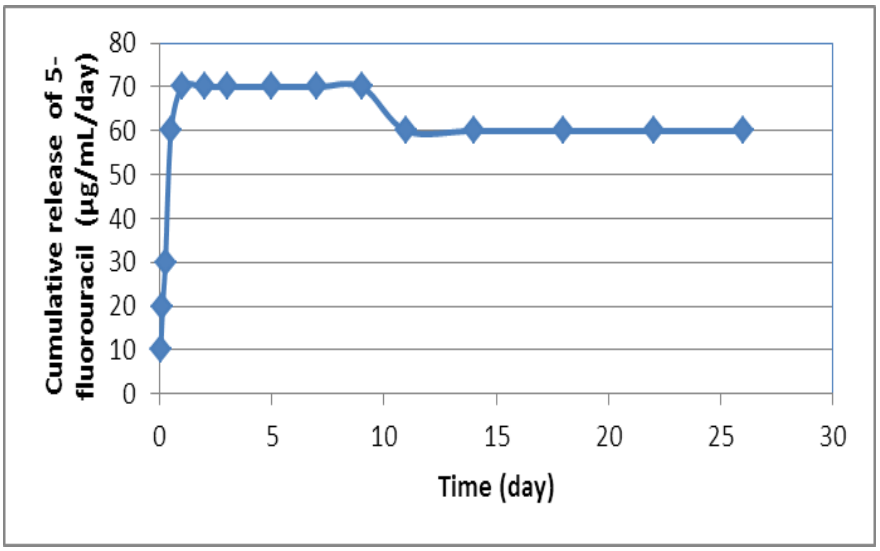

Fig. 2: Release profile of 5-fluorouracil from poly β- amino ester the in distilled water at R Temp. as determined from UV detection.

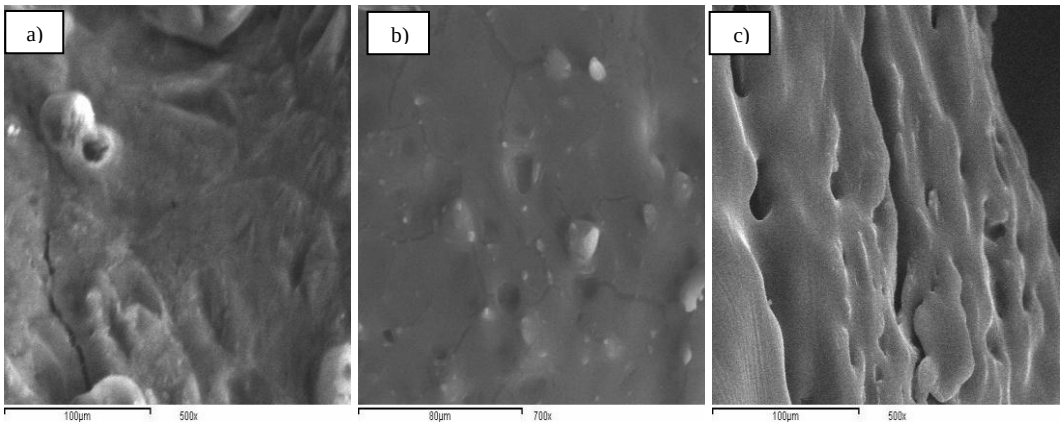

Fig. 3. SEM of surface polymeric textures a) without 5-fluorouracil, b)after loading with 5-fluorouracil, and c) after releasing 5-fluorouracil in distilled water for 3days.

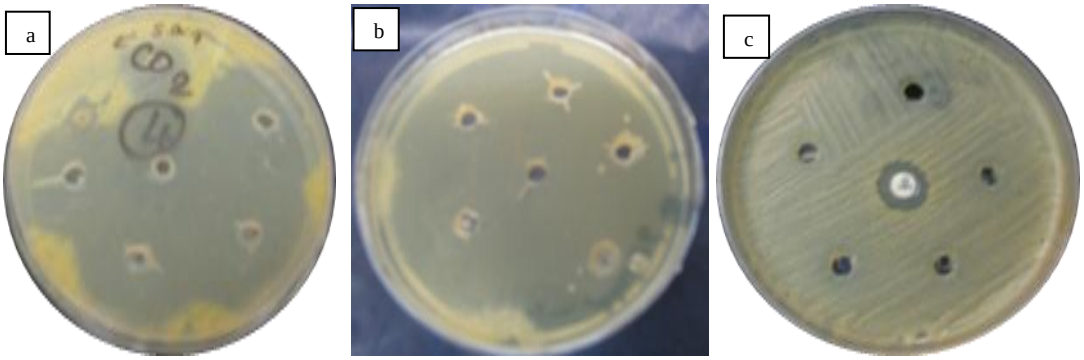

Fig. 4: *S. aureus* plate showing a) Samples: 1h, 3h, 6h, 12h, 24h and 48h were added in sequence in anticlockwise, b) Samples: 5d, 7d, 9d, 11d, 14d and 22d were added in sequence in anticlockwise, and c) DMSO (peripherally) and ANTIBIOTIC (AMC30) (center) were added.

CONCLUSIONS

The sustained release of the active agent showed that, the prepared poly β- amino ester is a promising polymeric biomaterial carrier for the antitumour drugs. The cytotoxic activity of the released 5-fluorouracil showed a sustained antiprolefrative activity against the tested human liver cancer cell line. Antimicrobial inhibitory activity was very promising against Enterotoxigenic *S.aureus* reference strain.

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