

## Evaluation of Slow Release System of Antitumor Bioactive organic compounds from Poly( $\beta$ -amino ester)

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### Research Article

#### Abstract

The present work is a trail to evaluate the synthesized slow release system of composed of poly poly( $\beta$ -amino ester) (PAE) containing drugs as antitumor for sustained time period. The network structures from poly( $\beta$ -amino ester) were synthesized *via* a simplified addition polymerization method to carry drug for applying as drug delivery matrix. It can hold the active organic compounds (drugs) that had an effect as antitumor activity in order to control their release. The active organic compounds that loaded into the prepared polymer matrix, are a new series of heterocyclic derivatives prepared from pyrimidine and naphthyridine namely: 7-(2-methoxy phenyl)-3-methyl-5-thioxo-5,6-dihydro[1,2,4] triazolo [4,3-c] pyrimidine-8-carbonitrile (D1), 7-(2-methoxy phenyl)-3-oxo-5-thioxo-2,3,5, 6-tetrahydro[1,2,4] triazolo [4,3-c] pyrimidine-8-carbonitrile (D2) and (*E*)-2-((furan-2-yl)methylene)-1-(2,7-dimethyl-1,8-naphthyridin-5-yl)hydrazine (D3).

The resulting polymer structures and the surface morphology of the PAE capsules before and after encapsulation with the active drugs were characterized by SEM. The SEM studies illustrate good dispersion and holding properties of the drug into the network structure of the prepared polymer. *In vitro*, the release results of the drug from the PAE capsules indicated that the capsules able to give sustained release of drug in DMF up to 15 days at 25°C. The polymer carried active compounds was tested for drug delivery through subjecting to release drug in aqueous media for different time periods and examined as anti-proliferative agents against human liver (HEPG2) cancer cell line.

Results showed that Compound (D2) gave the highest growth inhibition activity followed by compound (D1) while compound (D3) indicated the lowest activity against human liver (HEPG2) cancer cell line. The promising results were obtained from the reveal data resulted for the human liver cancer cell line.

**Keywords:** Poly( $\beta$ -amino ester), addition polymerization, drug delivery, swelling, degradation, *in vitro* release

#### Introduction

Cancer is a major public health problem in the world, which causes millions of death each year [1]. Many therapeutic anticancer drugs, while pharmacologically effective in cancer treatment, are limited in their clinical applications by serious toxicities. The world follows up the development of anticancer drug carriers to improve therapeutic efficacy and reducing unwanted side effects [2]. Cationic copolymers have been developed based on poly (amino urethan) and poly (amido-amine) [3,4].

Poly ( $\beta$ -amino ester) (PAE) is a cationic polymer which is easily synthesized by addition polymerization and known to be readily biodegradable of low cytotoxicity [5]. PAE acts as a hydrophilic block because of the ionization of tertiary amine at a relatively low pH and becomes a hydrophobic block because of deionization of tertiary amine at higher pH [6]. PAE has been widely investigated for gene

delivery [7] and cancer cytoplasmic drug release [8].

The pyrimidine moiety with some substitution shows promising antitumor activity as there are large numbers of pyrimidine based antimetabolites. The structural modification may be on the pyrimidine ring. Early metabolite prepared was 5-fluorouracil [9]. Pyrimidines occupy a distinct and unique place in our life. This heterocyclic moiety has great biological and medicinal significance. The biological profiles of this new generation of pyrimidine represent much progress with regard to the older compounds [10]. A pyrimidine derivatives followed by 5-Thiouracil which also exhibits some useful antineoplastic activities [11]. Thiouracils are also used as therapeutic agents for anticancer [12]. Synthesis of novel thiouracil derivatives had showed diverse biological activities of thiouracil as anticancer [13, 14, 15, 16,17].

Also, 1,8-Naphthyridine derivatives are well-known for their diverse biological activities including antitumor [18], anti-inflammatory [19], and antimicrobial activity [20]. In addition 1,8-Naphthyridine derivatives were found to display moderate cytotoxic activity against murine P388 leukemia cell line, when changes were carried out at N-1 and C-7 positions [21,22].

The present work aims to evaluate the efficiency of the synthesized poly ( $\beta$ -amino ester) containing the prepared active organic compounds as slow release system anticancer drug for sustained period of time through studies the release rate and bioassay on human liver cancer cell line.

## Materials and Methods

### Materials

Piperazine and 1,4-butanediol diacrylate were purchased from Sigma-Aldrich. N-hexane, dimethyl formamide (DMF), dimethyl sulfoxide (DMSO), dichloro methane (DCM), ethyl acetate (EtOAc) and tetrahydrofuran (THF) were obtained from Aldrich.

The rationales for the synthesis and characterization of the selected pyrimidine derivatives (D1 and D2) and naphthyridine derivative (D3) by elemental analysis, infrared, electronic spectra, room temperature magnetic measurements and powder X-ray diffraction were previously published by Fathallaa et al [23] and Eweas et al [24].

### Methods

#### Synthesis of Poly ( $\beta$ -amino ester)

The polymer synthesis was carried out according to Michael addition polymerization: from 1,4-butanediol diacrylate and piperazine [25].

#### Drug encapsulated polymeric capsules

The polymeric latex (1g) of PAE was mixed with (0.1g) of the active prepared drug (organic compound which had promising effect as antitumor) [26]. The drug polymer capsules were left to equilibrate for 24 hours at room temperature.

#### Determination of the drug encapsulation efficiency

The encapsulation efficiency (EE) was measured after extracting the drug from the prepared polymeric capsules<sup>1</sup>. 100 mg of drug-loaded polymeric capsules were subjected to disperse in 100 mL DMF and stirred for 30 min at 37°C to ensure the complete extraction of the drug. The

mixture was stirred magnetically at 1000 rpm for 4 h. The mixture solutions increased to 250 mL volumes with DMF. After centrifuging at 4000 rpm for 30 min, these solutions were diluted and analyzed by a UV-Vis spectrophotometer at 360 nm. All experiments were done in triplicate. The EE of drug is calculated and expressed as the following equation:

$$EE (\%) = \frac{\text{Practical drug loading}}{\text{Theoretical drug loading}} \times 100$$

#### Drug release from polymeric capsules

The procedure to determine the *in vitro* release of the drug from the polymeric drug-loaded capsules prepared was immersed in 10 mL DMF at 37°C with horizontal shaking. At timed intervals, 1 mL of the medium was withdrawn and the same volume of fresh DMF was added to keep the total volume constant. The drug released into the fluid was determined from the measurement of absorbance at 360 nm analyzed by a UV-Vis spectrophotometer according to the standard curve and expressed as follows:

$$\text{Drug release (\%)} = \frac{\text{released drug}}{\text{total drug}} \times 100$$

Where; released drug was calculated from the drug concentration (mol/L) measured in the total solution volume and total drug was the amount loaded in each capsule. All experiments were done in triplicate.

#### Characterization

The characterization of the prepared polymer before and after loading with the organic compounds was carried out according to the following;

Surface morphology was visualized by scanning electron microscopy (JXA-840A Electron probe microanalyzer, JEOL, Japan) using an accelerating voltage of 30 KV after coating with gold film using S150A Sputter Coater (Edwards, England).

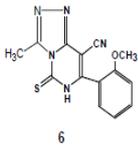
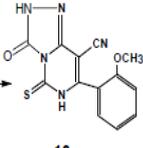
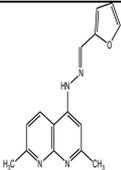
The drug loading and release studies were measured using double-beams Spectrophotometer (Shimadzu UV-2401 PC, Japan).

#### Anticancer testing

##### Measurement of potential cytotoxicity by sulforhodamine B (SRB) assay

The active organic compounds released from the polymeric system were subjected to a screening system for evaluation of their antitumor activity against HEPG2 Liver cancer cell line [27].

**Table 1: Specifications of the new synthesized active compounds**

No.	Name	Chemical Structure	Molecular formula/weight	Color / shape	Melting point (°C)
D1	7-(2-methoxyphenyl)-3-methyl-5-thioxo-5,6-dihydro[1,2,4]-triazolo [4,3-c] pyrimidine-8 carbonitrile		C <sub>14</sub> H <sub>11</sub> N <sub>5</sub> O S, Mw(297.33)	black /crystals	200-202
D2	7-(2-methoxyphenyl)-3-oxo-5-thioxo-2,3,5,6-tetrahydro[1,2,4] triazolo[4,3-c]pyrimidine-8-carbonitrile		C <sub>13</sub> H <sub>9</sub> N <sub>5</sub> O <sub>2</sub> S, Mw(299.30)	pale green/crystals	195-197
D3	(E)-2-((furan-2-yl)methylene)-1-(2,7-dimethyl-1,8-naphthyridin-4-yl)hydrazine		C <sub>15</sub> H <sub>14</sub> N <sub>4</sub> O, Mw266.3	Yellow/crystals	238-240

## Results and Discussion

### Poly (β-amino ester)

PAE was used as a carrier for some bioactive organic compounds which were indicated in Table 1

### SEM characterization

It is interesting to illustrate the surface texture of the investigated Poly (β-amino ester) before and after carrying the drug. Scanning electron microscope is a valuable technique for this purpose. Fig. 1 illustrates the micrograph of the surface texture of the prepared products. It was shown that, a good homogeneity for the surface texture and good dispersion and penetration of the drug through the surface texture of the polymer were obtained. So, it is recommended that Poly (β-amino ester) was a good polymer carrier for drug.

### Encapsulation and controlled release of drugs from PAE

The percentage of encapsulation efficiency of the drugs-loaded PAE was 100%. The potential application of PAE polymer for drug delivery was assessed by examining the *in vitro* release of D1, D2 and D3, anticancer drugs, from this polymer. The release rate of D1, D2 and D3 from PAE was evaluated spectrophotometrically at 360, 310 and 330 nm, respectively within the first 48h as shown in Fig. 2. It was found that PAE polymer released the anticancer drug in a

slow manner. Furthermore, the release profiles showed that active compound drugs had undergone controlled release from PAE polymer for more than 10 days.

### Biological efficiency on liver cancer of cell line

Table 2 showed that the efficiency of drug released from the polymer formulation on growth inhibition of HEPG2 liver cell line is depend on time as the longer the time of sustained release of the drug the higher the growth inhibition activity. Results also indicated that compound D2 gave the highest growth inhibition potency followed by compound D1 while compound D3 showed the lowest activity.

### Conclusions

Network structure of poly (β-amino ester) was synthesized from piperazine and 1,4-butanediol diacrylate by Michael addition polymerization. The prepared polymer carried drug characterized by FT-IR and SEM. The release of the drug was extended to over 10 days in DMF at 25°C. The present work indicated that the cytotoxicity of the released drug against HEPG2 liver cancer cell line is dependent on time as the longer the time of sustained release, the higher the growth inhibition potency of the drug.

Table 2: Drug Cytotoxicity against HEPG2 Cell Line

Compound	Survival %				Inhibition %			
	30min	6hr	24hr	One week	30min	6hr	24hr	One week
D1	40	39	38	25	60	61	62	75
D2	37	37	35	22	63	63	65	78
D3	47	44	41	28	53	56	59	72

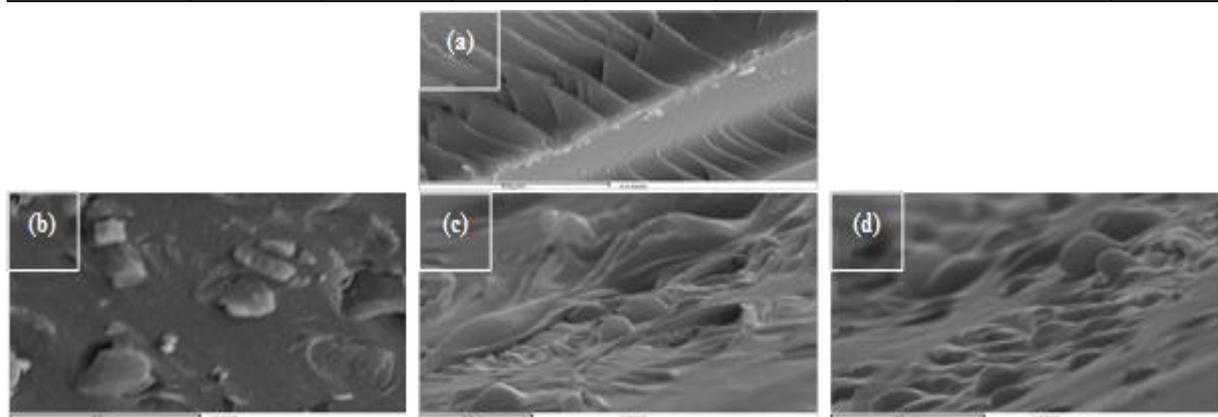


Figure 1. SEM of PEA (a) before and (b) after loading with D1, (c) after loading with D2, (d) after loading with D3.

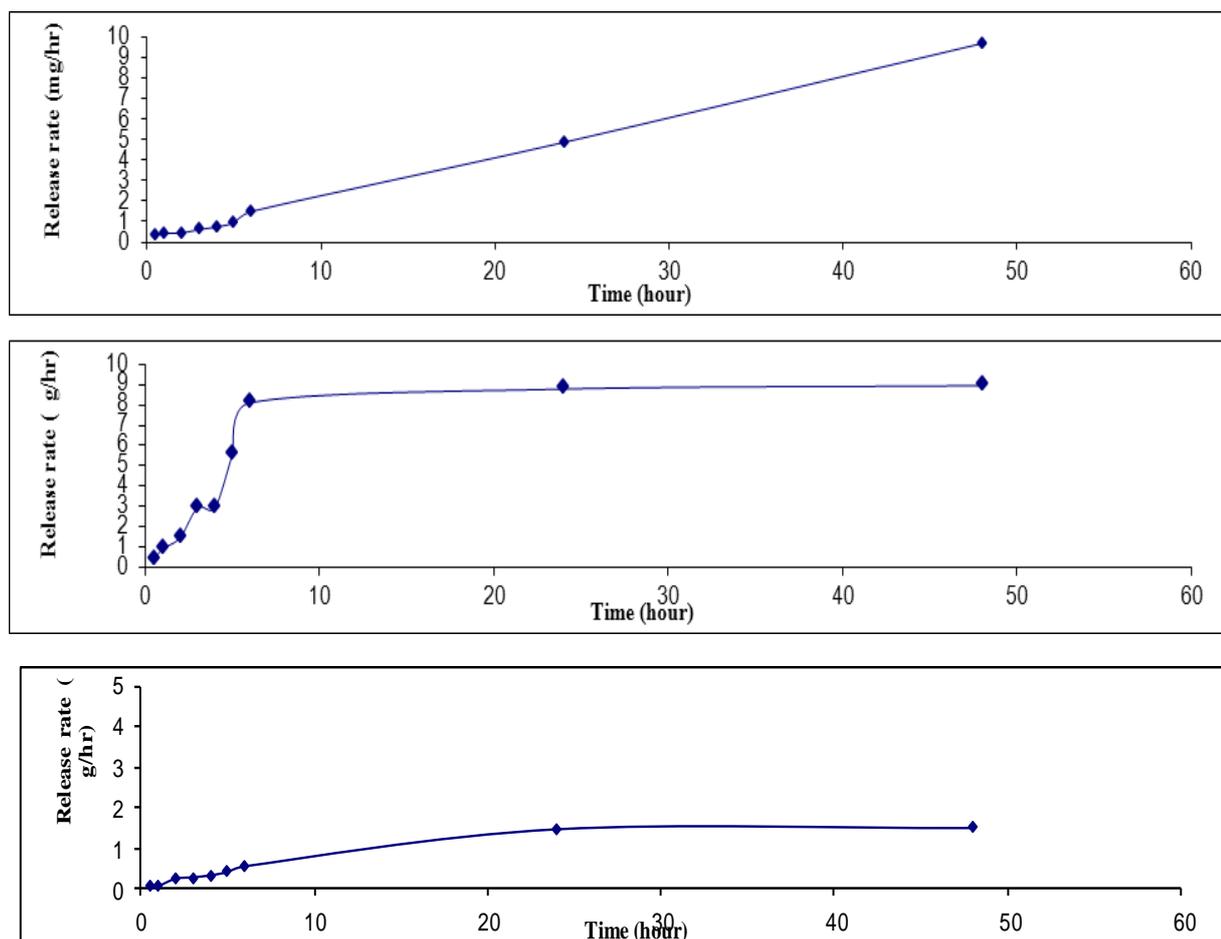


Figure 2. The release rate of D1, D2 and D3 from PAE in DMF.

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