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Assessment in vitro Controlled Release Polymeric Nanocomposites Material Loaded with Different Drugs

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SYNTHESIZED starch cellulose acetate coacrylate (SCACA)/ Sodium bentonite modified Tween-80 (MSB) nanocomposites holding anticancer drugs like 5-fluorouracil (5-FU), Doxorubicin hydrochloride (DOX), and Chlorambucil (CA) as controlled release systems were evaluated and assessment as antiproliferative activity towards breast cancer cell lines, and antimicrobial activity against some strains. The nanocomposites loaded drugs characterized by Zeta Potential, FTIR, SEM and TEM. The results indicated that no change in the chemical structures and physical properties of the drugs into the nanocomposites. The surface texture of the samples was homogenous and smooth with no evidence of aggregations after release and there were some pore like structure and cracks formed due to blooming of drug outside nanocomposites. The particle size diameter of the prepared polymer was found to be 73 ~ 79 nm. After holding Doxorubicin the particle size was from 18 to 24 nm , 5-fluorouracil from 15 to 18 nm and Chlorambucil was from 24 to 75 nm. The release of drug was measured spectrophotometrically. The values of drug released depend on the type and nature of drug as well as the environmental aqueous media. The release in alkaline and acidic media was more faster and higher than that in the neutral one.

The invitro study results of anticancer drugs released from polymers placed inside the culture media, showed 5-FU was given an effective promising results compared to DOX and CA. The antimicrobial test revealed that, the released 5-FU gave the highest effect as antimicrobial against all tested strains followed by DOX and CA.

Keywords: Controlled release, Polymeric nanocomposites, Drugs, Breast cancer, Antimicrobial.

Introduction

Controlled release polymeric drug delivery system is a new trend for treatment some serious diseases in order to achieve appropriate therapeutic effect in the human body. it is the key objective of slow release drug delivery technique[1].

The polymeric matrices provide remarkable protection to the incorporated active compounds against thermo-or photo degradation. The biopolymer-based nanoparticles have drawn increasing interest as a vehicle for the protection and delivery the bioactive compounds [2].

The nano scale size contributes to higher solubility, better tissue permeability, prolonged

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clearance time and improved cellular uptake of the entrapped compounds [3,4].

Polysaccharides are the major category of biopolymers that had many applications for food and pharmaceutical industry[5].

Recently starch cellulose acetate coacrylate containing different anticancer drugs as 5-fluorouracil (5-FU) [6, 7] gemcitabine, Doxorubicin (DOX) [5], Chlorambucil (CA) ---- etc. were prepared for sustained controlled release system [8-10]. It is applied as a new trial for long acting antitumor. It is applied as a new trial for long acting [7] antitumor.

The sustained release drug delivery systems are the unique means which are categorized by the quick and unhindered drug release rate and release kinetics [2, 11]. Drug delivery at controlled rate is attractive method and pursued vigorously due to its special biological characteristics, consisting of biodegradability and low toxicity[12].

Cancer is a genetic disease characterized by abnormal cell growth, proliferation and metastasis that leads to high mortality. Doxorubicin is a DNA intercalating agent used[13].

The aim of the present work is to evaluate and assessment the in-vitro controlled release nanosized starch cellulose acetate coacrylate containing different anticancer drugs as antiproliferative activity for breast cancer cell lines as well as their effect as antimicrobial activity for some strains.

Materials and Experimental Techniques

Materials

- Potato starch was supplied as neutral white powder by El Nasr Pharma Central Chemical Company, Abu Zaabal, Egypt.
- Cellulose acetate containing 40 % acetyl group was supplied by Sigma-Aldrich.
- Acrylic acid with molecular weight of 72, freezing point of 13 °C, boiling point of 141 °C, density at 20 °C of 1.046 g cm^{-3}, and refractive index at 25°C is 1.4185 was supplied by Sigma-Aldrich.
- Ethyl alcohol with density of 0.789 g cm^{-3} and boiling point of 78 °C was supplied by Aldrich Company, Germany.
- Sulfuric acid with density 1.84 g cm^{-3} and boiling point 337 °C was supplied by Aldrich Company, Germany.
- Sodium hydroxide pellets are odorless, white, solid hemispheres of uniform diameter and thickness with melting point 318.4 °C. It was supplied by Sigma-Aldrich.
- Dicumyl peroxide (DCP), pure grade, melting point (39-41^{o}C) Mwt = 270.37g/mol, Sigma-Aldrich product.
- Sodium bentonite clay powder (mesh size 300 mm), with a cationic exchange capacity (CEC) of 90 ml equiv. per 100 g.
- Tween-80 (polyethylene sorbitol ester) non-ionic viscous liquid and average molecular weight 1,310 Da purchased from Sigma-Aldrich.
- 5-fluorouracil (5-FU),2,4-Dihydroxy-5-fluoropyrimidine, assay is 99% with melting point of 282-286 °C by Sigma-Aldrich.
- Doxorubicin hydrochloride (DOX), Mwt= 579.98 g/mol , mp= 216 °C and empirical formula: C27H29NO11 · HCl, Sigma-Aldrich product.
- Chlorambucil (CA), 4-(4-[Bis(2-chloroethyl) amino]phenyl)butyric acid, 4-[Bis(2-chloroethyl)amino] benzenebutyric acid, Molecular Weight 304.21.

Instruments

Ultra Violet Spectroscopy

The amount of drug released was determined spectrophotometrically. The spectrophotometer used was a UV-240 1PC Visible VIS.

Morphology Study.

A-The morphologies of the prepared controlled release samples were investigated using scanning electron microscopy (SEM, Quanta FEG 250, FEI). To prepare the SEM sample, a thin layer of Au was coated onto the specimens.

B- High-Resolution Transmission Electron Microscopy (HRTEM). The average particle sizes of materials and synthesized polymer were measured using a (HRTEM). JEOL JEM 2100 T.E.M HR Japan.

Dynamic light scattering (DLS)

Dynamic light scattering (DLS) instrument (PSS, Santa Barbara, CA, USA)[NICOMP 380 ZLS] , using the 632 nm line of a He Ne laser as the incident light with angel 90^{o} and Zeta potential with external angel 18.9^{o}. nanomaterial investigation lab., Central laboratory, national research Centre.

Fourier-Transform Infrared (FTIR) Spectroscopy

The FTIR spectra of samples were obtained using a Jascow (Japan) FTIR 430 series infrared spectrophotometer equipped with KBr discs.

Experimental Techniques

Preparation of Polymer/Clay nanocomposites loaded with different drugs [14]:

This process including,

a) Modification of clay by distilled water.

b) Treatment with non-ionic surfactant (Tween-80) .

c) Preparation of SCACA/modified nano-clay loaded with different drugs.

Leaching rate detection:

The leaching rate technique was used similar to that described by Marson.[15]. The synthesized starch cellulose acetate co-acrylate (SCAA)/ Sodium bentonite modified Tween-80 (MSB) nanocomposites loading drugs, 5-fluorouracil (5-FU), Doxorubicin hydrochloride (DOX) and Chlorambucil (CA) was used in the form of circular discs; radius 0.75 cm and height 0.5 cm and total surface area ~ 5.89cm^2. The samples immersed in 50 ml aqueous media of different pHs; 7.5, 4 and 9 at room temperature. The leachable medium was changed daily during the test period and the released drug was measured spectrophotometrically. The applied wave length was 222, 450 and 258 nm for (5-FU), (DOX) and (CA) respectively.

1.5. Invitro testing for the toxicity of anticancer drugs released from the prepared polymeric nanocomposites towards cancer and normal cell lines.

A- Cells:

Cell lines: MCF-7 (human breast cancer) and BALB/3T3 (murine fibroblast) were obtained from American Type Culture Collection (Rockville, Maryland, USA) and are being maintained in the Ludwik Hirszfeld Institute of Immunology and Experimental Therapy (Wrocław, Poland). MCF-7 cells were cultured in Eagle medium (IIET, Wroclaw, Poland) supplemented with 2 mML-glutamine, 10% fetal bovine serum, 8 µg/mL of insulin and 1% mem non-essential amino acid solution 100x (all from Sigma–Aldrich Chemie GmbH, Steinheim, Germany). BALB/3T3 cell line was cultured in DMEM (Gibco, UK) supplemented with 2 mML-glutamine, 10% fetal bovine serum (GE Healthcare, Logan, UT, USA). All culture media were also supplemented with antibiotics: 100 µg/ml streptomycin (Sigma–Aldrich Chemie GmbH, Steinheim, Germany) and 100 units/ml penicillin (Polfa Tarchomin SA, Warsaw, Poland). All cell lines were grown at 37 °C with 5% CO_2 humidified atmosphere.

B- Released anticancer drugs:

Anticancer drugs released from polymer in distilled water:

The synthesized polymer of starch, cellulose acetate coacrylate (SCAAC)/ Sodium bentonite modified Tween-80 (MSB) nanocomposites loaded with different anticancer drugs such as 5-fluorouracil, doxorubicin, and Chlorambucil, were subjected to distilled water and put in tubes of ultrasonic bath (Ultrasonic Cleaner, SH80,USA) for 30 min. Ice was added to maintain the deionized water at room temperature during the indirect sonication treatments for releasing the active drugs over different time periods.

Anticancer drugs released from polymers in culture media:

Prior to usage, the polymers binding drugs were stored in room temperature and subjected to release the investigated drugs into culture media according to proportion of 5 mg per 20 ml and. Samples of culture media containing released compounds were collected after 1, 7, 14 and 21 days and also stored in room temperature.

Prior to usage, the corresponding free drugs were dissolved in culture media to the concentration of 0.250 mg/ml and subsequently diluted in culture medium to reach the required concentrations (ranging from 10 to 0.01 µg/ml in case of Doxorubicin (DOX), and 5-fluorouracil (5-FU) and ranging from 125 to 1 µg/ml in case of Chlorambucil (CA).

C- Antiproliferative assay in vitro

24 hours before addition of the tested drugs, both free and bound by polymers, the cells were plated in 96-well plates (Sarstedt, Germany) at density of 1x10^4 cells per well. The assay was performed after 72 hours, exposure to varying concentrations of the tested drugs. The in vitro cytotoxic effect of all anticancer drugs was examined using the SRB assay.

D- Cytotoxic test: The sulforhodamine B (SRB) assay

The details of this technique were described by Skehan et al. The cells were attached to the bottom of plastic wells by fixing them with cold 50% TCA (trichloroacetic acid, Sigma-

Aldrich Chemie GmbH, Steinheim, Germany) on the top of the culture medium in each well. The plates were incubated at 4°C for 1 hour and then washed five times with tap water. The cellular material fixed with TCA was stained with 0.4% sulphorhodamine B (SRB, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) dissolved in 1% acetic acid (POCH, Gliwice, Poland) for 30 minutes. Unbound dye was removed by rinsing (fife times) in 1% acetic acid. The protein-bound dye was extracted with 10 mM unbuffered Tris base (POCH, Gliwice, Poland) for determination of the optical density (λ = 540 nm) in Synergy H4 multi-mode micro plate reader (BioTek Instruments USA).

E- Antimicrobial activity

Antimicrobial activities of the different time intervals released drugs (Doxorubicin, Chlorambucil and 5-fluorouracil) from prepared polymers were in vitro evaluated againsta panel of Gram positive [16] bacteria: Staphylococcus aureusATCC 29213, Bacillus subtilis ATCC6633; Gram negative bacteria: Escherichia coli ATCC, Pseudomonas aeruginosa ATCC27953 and fungi: Candida albicans NRRL Y-47

F- Antimicrobial tests

Antimicrobial tests were carried out by the agar well diffusion method [17]. using 100 µL of suspension containing 1 x108 CFU/mL of pathological tested bacteria ,1 x106 CFU/ml of yeast spread on nutrient agar (NA) and Sabourand dextrose agar (SDA) respectively. After the media had cooled and solidified, wells (10 mm in diameter) were made in the solidified agar and loaded with 100 µL of tested solution. The plates were then incubated for 24 h at 37 °C for bacteria and 48h. After incubation time, antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms and compared with that of the standard.

Results and Discussion

Characterization of the investigated polymers loaded drugs

FTIR analysis

Figure (1a-d) shows the IR spectra of the prepared SCACA and SCACA/MSB containing different drugs such as doxorubicin, 5-Fluorouracil and chlorambucil nanocomposites. In Figure (5a) the characteristic absorption bands for the functional groups of the prepared SCACA are found at 3439 cm^{-1} for OH group, 1740 cm^{-1} , 1650 cm^{-1} for carbonyl group in acetate and acrylate groups respectively.[18] An overlay of FTIR of the prepared SCACA / MSB /drugs nanocomposites is shown in Figure (5b-d) the same characteristic absorption bands of SCACA beside the characteristic absorption bands of clay and drugs. The spectrum of the clay shows a broad absorption band at 3639–3458 cm^{-1} corresponding to the OH stretching vibration of water in the interlayer space of the clay. In addition, a sharp band corresponding to the Si-O-Si stretching vibration of the layered silicate is observed at 1009-1024 cm^{-2} and the Si-O-Si and Al-O bending vibration bands are located at 573 and 444–424 cm^{-1} .[19] Moreover, FTIR spectra of polymer/clay/drugs nanocomposites showed more additional absorption bands compared with that of the neat clay. The spectra of SCACA/ MSB / DOX nanocomposites (Figure 1b) show the absorption bands of 3750–3100 cm^{-1} due to N–H stretching vibrations for the primary amine structure and 2924 cm^{-2} for C–H stretching vibrations. Also, the bands observed at 858 cm^{-1} due to the presence of N–H was in DOX. Figure (1c) illustrates the characteristic absorption bands for the functional groups which belongs the 5-FU: broad NH for primary amine at about 3443 cm^{-1}, C=O at 1684cm^{-1}, amide group at 1733 cm^{-1} and C–F at 1170 cm^{-1}. On the other hand Figure (1d) illustrates the characteristic peaks for the functional groups which belongs the chlorambucil: C=C (aromatic) at 1428 cm^{-1}, C=O (carboxylic) at 1716 cm^{-1} and C–F at 737 cm^{-1}.
Scanning Electron Microscopy (SEM) 2-

Figure 2(a-d) illustrated the of the investigated polymer carrier loaded clay/ tween as surfactant and drugs; 5-fluorouracil (5-FU) , Doxorubicin hydrochloride (DOX) and Chlorambucil (CA); it was found that the surface texture of the tested samples are homogeneous and had a good distribution of all ingredients, also smooth with no evidence of aggregations.

Figure 3 shows the SEM image of SCACA/ MSB / 5-fluorouracil after subjecting to the release media of pH 9 for 20 days.

It was observed that, some pore like structure and cracks were formed. This may be due to the release of drug outside the polymer carrier according the diffusion dissolution mechanism. [14] The dimension of pore are 2.67x4.21x1.5 µm.

Transmission electron microscopy (TEM)

It is important to provide some information about the particle size diameter of the prepared

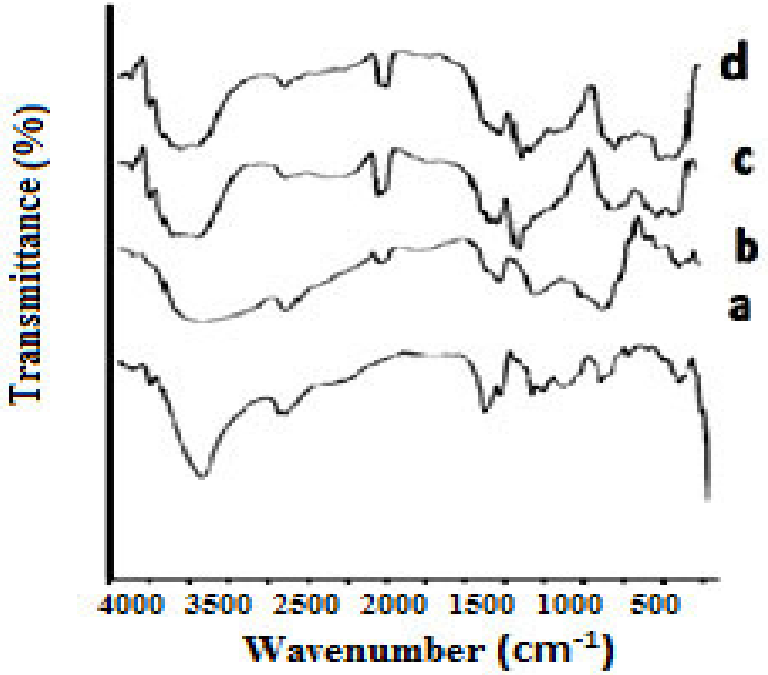

Fig. 1. FTIR spectra of:
(a) SCACA (b) SCACA/MSB/5-fluorouracil (5-FU) (c)SCACA/MSB/Doxorubicin (DOX)
(d) SCACA/MSB/Chlorambucil (CA)

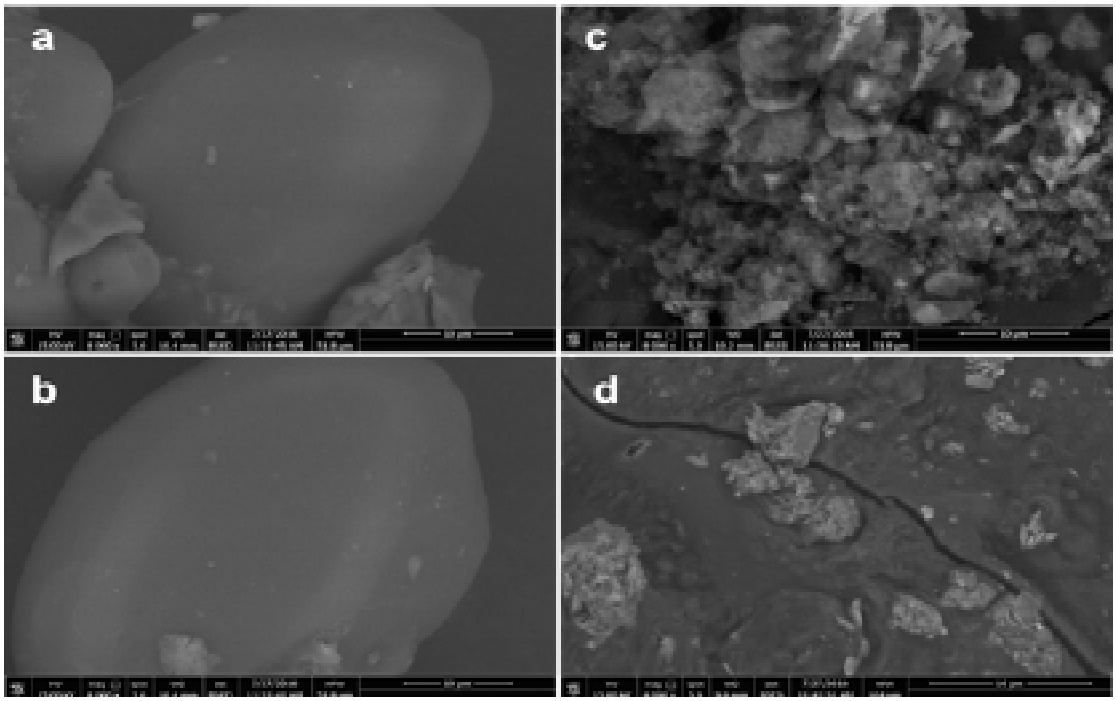

Fig. 2. Scanning electron microscope:
(a) SCACA/MSB (b) SCACA/MSB/ 5-fluorouracil (5-FU) (c) SCACA/MSB/ Doxorubicin (DOX)
(d) SCACA/MSB/Chlorambucil (CA)

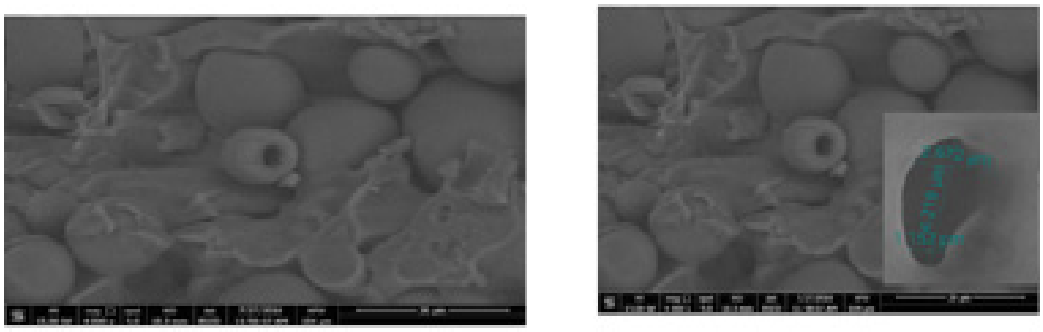

Fig. 3. Scanning electron microscope of SCACA/SB/tween/ 5-fluorouracil after subjecting to the release media of pH 9 for 20 days.

investigated nano-sized controlled release polymeric materials containing drugs. This was done and estimated by Transmission electron microscope (TEM) technique. Figure 4 (a,b,c,d) illustrate the particle size of the prepared polymer before and after loading drugs. It was shown that, the particle size diameter of SCACA/SB/tween was found to be from 14 to 146 nm; for SCACA/SB/tween/ 5-Fluorouracil (5-FU) was from 6.79 to 19 nm. For the SCACA/ SB/tween/ Doxorubicin (DOX) the particle size range from 18 to 24 nm and for SCACA/ MSB / Chlorambucil (CA) the particle size range from 24 to 39 nm. The variation of the values of the nano sized depending on to the type and the nature of material applied.[20,21]

Zeta Potential Study

Zeta potential is a significant parameter representing the stability of drug particles measured, i.e. not change in chemical structure or physical modification of drugs (5-FU), (DOX) and (CA). So, it is interesting to examine the Zeta potential of the investigated drug particles after releasing from the synthesized SCACA/ MSB loaded with (5-FU), (DOX) and (CA).[22] The results are summarized in Table 1.

It was found that, the particles of various investigated drugs (1,2) displayed a Zeta potential at about-18.83, -20.24 and -18.64 mv. These results indicated no change in the chemical structure of drugs after incorporated into the synthesized polymer (SCACA). Also, no physical modification observed on the drugs that released in distilled water.

The obtained drug mean, particle size, was arranged as the following order:

5-FU> DOX > CA

And the poly dispersed index (PDI) was observed on the opposite order direction as follows

CA>DOX >5-FU

Release Profile of drug released from SCACA study:

The results of the leaching rates of the active compounds in distilled water at 25 ^{o}C expressed as μg/mm^2/day are represented in Figure 5. It was found that, the highest amount of release was observed in the initial stage releasing process that observed after 48 h for compound 5-FU and 72 h for compounds Dox and CA, then the amount of release decreased and steady state sustained release was obtained up to the period of study of 480 h. [23] The release rate was changed according to the type of the investigated drugs which depends on the structure and the nature of the active compounds, as well as the particle size distribution due to polydispersity index.

The release rate of the detected active compounds in the steady state can be arranged according to the following order:

5 Flu $\simeq$ Dox > CA

This results encourage to study the release of drug in aqueous media at different pHs. (alkaline and acidic)

Effect of pH of aqueous media on the release of drugs:

It is important to study the release behavior of drugs in acidic and basic aqueous medium to simulate the gastric and intestinal pH conditions, in order to regulate the drug delivery for anticancer activity.

There for samples (discs) of the prepared starch cellulose acetate coacrylate /MSB loaded

with drugs; 5-fluorouracil (5-FU) , Doxorubicin hydrochloride (DOX) and Chlorambucil (CA) were subjecting to release drugs in the specific media at room temperature and the released drugs were examined for different time periods. The release was measured at pH 9 for the basic medium and pH 4 for acidic medium . [24] The results were represented on Figures(6,7).It was found that, the release of drugs at the initial stage of release (before 5 days) the release was higher than that in the steady state The sustained slow release drugs were extended to about 45 day for all drugs under investigation and the amount of drug released depends on the pH of aqueous media and the solubility of drugs. Also it was shown that, in the basic medium, the release of Doxorubicin was higher than Chlorambucil and 5-Fluorouracilas the following order.

Doxorubicin > Chlorambucil > 5-Fluorouracil.

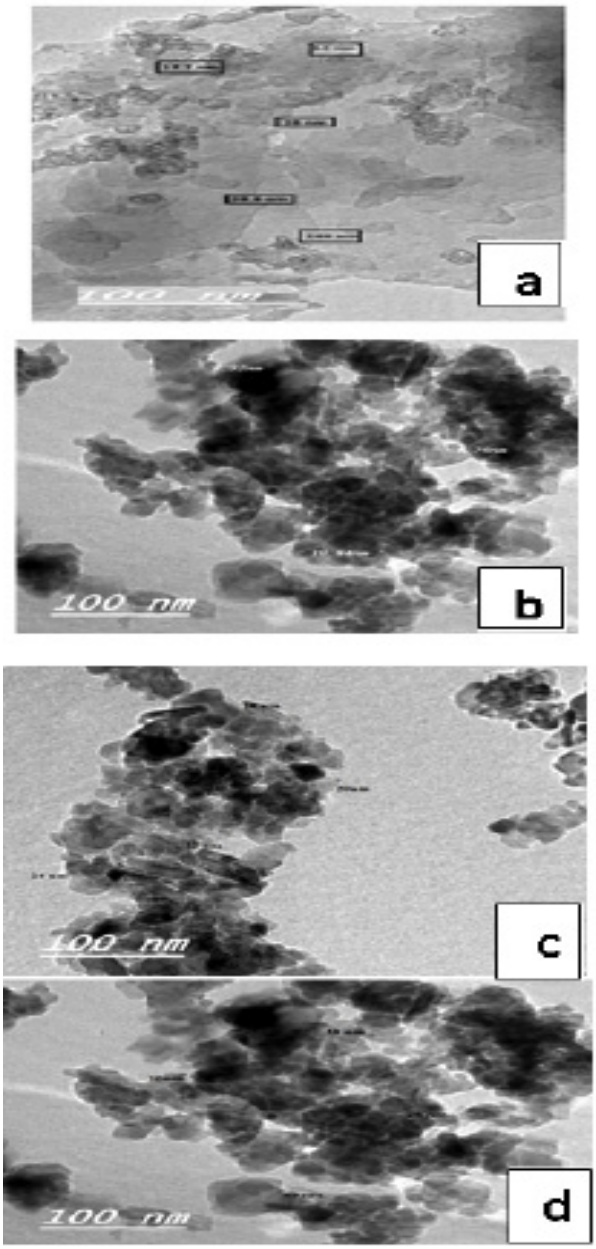

Fig. 4. Transmission electron microscope (TEM):
(a) SCACA/MSB (b) SCACA/MSB/ 5-fluorouracil (5-FU) (c) SCACA/MSB/ Doxorubicin (DOX) (d) SCACA/MSB/Chlorambucil (CA)

TABLE 1. The mean particle size, poly dispersed index (PDI), standard deviation and Zeta potential of the tested release drugs.

SCACA/MSB loaded with	Mean particle size	Poly dispersed index (p.d.i)	Standard deviation	Zeta potential, mv
5-FU	150μ	4.567	215.8%	-18.83
DOX	59μ	7.806	279.4%	-20.24
CA	49.6μ	11.806	343.6%	-18.64

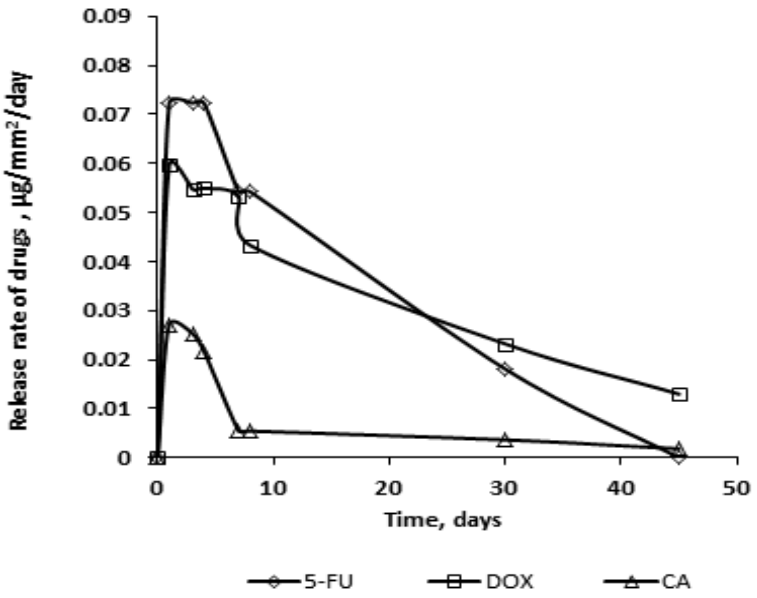

Fig. 5.The release rate of (5-FU) , (DOX) and (CA) from the investigated SCACA/MSB in distilled water

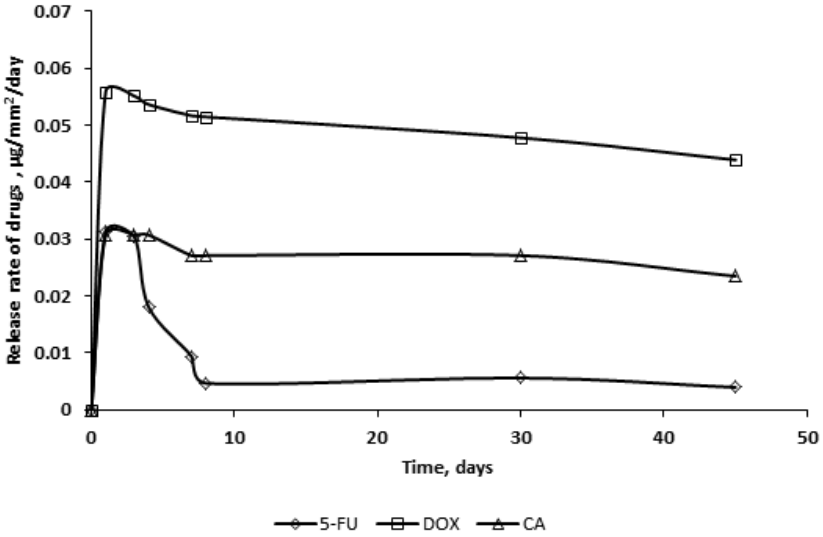

Fig. 6. The release rate of (5-FU) ,(DOX) and (CA) from the investigated SCACA/MSB in alkaline at pH 9.

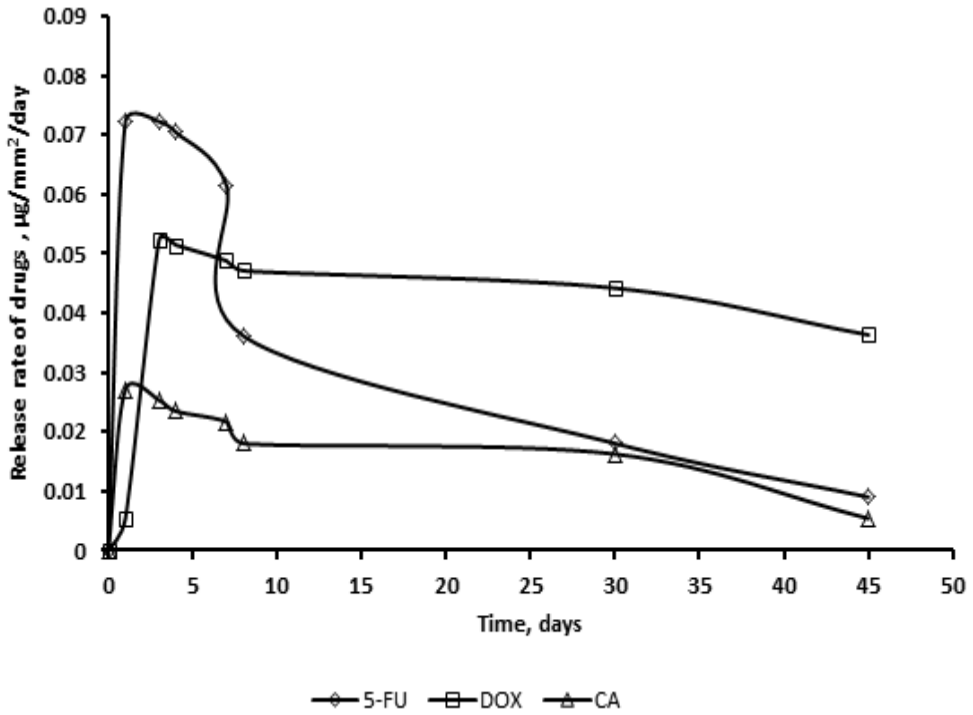

Fig. 7.The release rate of (5-FU) , (DOX) and (CA) from the investigated SCACA/ MSB in acidic at pH 4

In acidic medium the release of Doxorubicin was higher than 5-FU of Chlorambucil as the following order:

Doxorubicin > 5-Fluorouracil > Chlorambucil

These results may be used as a guide line for selecting the proper effective release suitable for specific purposes.

5.Invitro testing for the toxicity of anticancer drugs released from the prepared polymer in water and in culture media towards different cancer and normal cell lines.

The results were calculated as the antiproliferative activity of tested anticancer drugs released from polymers in designated time points (1, 7, 14 and 21 days after the polymers binding tested drugs were subjected to culture media) and stated as percentage of proliferation inhibition.

Percentage of proliferation inhibition of cells human breast cancer MCF-7 and murine fibroblast BALB/3T3 by drugs released from the prepared polymer clay nanocomposites and also free drugs dissolved in culture media used as positive controls was calculated for each experiment separately and mean values ± SD are presented in the tables presented below. Each drug at each concentration as well as each sample of drugs released from polymers in water and in culture media, was tested in triplicate in a single experiment, which was repeated 3 times. The obtained results of the studies are summarized in Tables 2 – 5.

The results showed that, the antiproliferative potency of the investigated SCACA/MSB polymer nanocomposites loaded with the anticancer drugs 5-FU, DOX and Chlorambuciltowardbreast cancer cell line MCF-7 as well as normal cell line BALB/3T3 was around 80% at first days, followed by slight decreased inhibition over the next 21days (Tables 2 and 4).On the other hand, in vitro studies results of anticancer drugs released from polymers in culture media indicated that only 5-FU is released from polymers compared to other polymers binding doxorubicin or chlorambucil. Almost whole drug is released at the first day after subjecting the polymers binding 5-FU to culture media. The percentages of proliferation inhibition of MCF-7 and BALB/3T3 cells at first day were 30.71 and 60.62, respectively. Antiproliferative activity of released 5-FU from polymers only slightly increased in subsequent time points (7, 14 and 21 days after subjecting the polymers binding 5-FU to culture media). In case

TABLE 2. Percentage of proliferation inhibition values of breast cancer cell line MCF-7 by drugs released from the investigated polymer nanocomposites in water

Release time (days)	doxorubicin released from polymer	chlorambucil released from polymer	5-FU released from polymer
1	80,47 ± 1,4	78,90 ± 2,0	79,56 ± 2,2
7	80,81 ± 1,7	79,59 ± 1,5	76,88 ± 0,5
14	78,984	79,64 ± 2,3	77,83 ± 2,2
21	77,895 ± 2,3	79,95 ± 2,6	77,68 ± 3,3

TABLE 3. Percentage of proliferation inhibition values of breast cancer cell line MCF-7 by drugs released from the investigated polymer nanocomposites and also by free drugs dissolved in culture media used as positive controls.

MCF-7

Proliferation inhibition [%] ± SD (n=3)

Tested drugs released from polymer

Release time [days]	Doxorubicin	5-FU	Chlorambucil
1	1.37 ± 0.37	34.71 ± 9.01	0.15 ± 3.64
7	1.78 ± 3.73	39.96 ± 14.58	-1.72 ± 2.45
14	0.15 ± 2.26	38.90 ± 16.58	-2.77 ± 3.77
21	0.49 ± 3.70	39.81 ± 21.26	-0.59 ± 0.99

Tested drugs dissolved in medium

Concentration [µg/ml]	Doxorubicin	5-FU	Concentration [µg/ml]	Chlorambucil
0.01	6.43 ± 5.36	1.22 ± 1.46	1	-0.23 ± 1.96
0.1	27.15 ± 4.85	0.21 ± 5.79	5	3.35 ± 6.88
1	72.74 ± 19.74	40.38 ± 5.83	25	7.02 ± 1.26
10	83.28 ± 8.74	60.13 ± 2.89	125	22.61 ± 7.97

TABLE 4. Percentage of proliferation inhibition of normal cell line BALB/3T3 by drugs released from polymer nanocomposites in water.

release time (days)	doxorubicin released from polymer	chlorambucil released from polymer	5-FU released from polymer
1	80,26	78,525	85,9
7	80,998	79,783	83,731
14	77,05	79,523	82,473
21	80	79,436	80,607

TABLE 5. Percentage of proliferation inhibition of normal cell line BALB/3T3 by drugs released from the investigated polymer nanocomposites and also by free compounds dissolved in culture media used as positive controls.

BALB/3T3				
Proliferation inhibition [%] ± SD (n=3)				
	Tested compound released from polymer			
Release time [days]	**Doxorubicin**	**5-FU**		**Chlorambucil**
1	-15.16 ± 1.90	60.62 ± 13.94		-17.06 ± 13.84
7	-24.12 ± 5.71	65.26 ± 4.75		-23.00 ± 17.65
14	-21.89 ± 6.90	64.12 ± 3.62		-17.63 ± 7.05
21	-14.07 ± 2.37	66.17 ± 6.09		-10.88 ± 10.71
	Tested compound dissolved in medium			
Concentration [µg/mL]	**Doxorubicin**	**5-FU**	**Concentration [µg/mL]**	**Chlorambucil**
0.01	15.98 ± 10.73	-3.85 ± 12.25	1	13.48 ± 3.92
0.1	34.99 ± 2.12	17.95 ± 18.86	5	24.24 ± 8.72
1	58.16 ± 18.86	61.89 ± 10.44	25	43.75 ± 14.01
10	75.68 ± 14.48	65.59 ± 7.06	125	70.32 ± 6.27

TABLE 6. Antimicrobial activity expressed as inhibition diameter zones in millimeters (mm) of the investigated polymer nanocomposites against the pathological strains based on diffusion assay

Chemical compound	**Time days**	**Staphelococcus aureus ATCC 29213**	**B. subtilis ATCC6633**	**E. coli ATCC 2592**	**Candida Albicans NRRL Y-477**	**Pseudomonas. aeroginosa ATCC27953**
Doxorubicin	1	14	15	13	12	N.A.
Doxorubicin	7	14	15.	13	12	N.A.
Doxorubicin	14	30	28	31	30	N.A.
Doxorubicin	20	14	15	15	13	N.A.
Chloroumbicl	1	13	15	13	12	N.A.
Chloroumbicl	7	14	15	13	12	N.A.
Chloroumbicl	14	30	28	31	30	N.A.
Chloroumbicl	20	14	15	15	13	N.A.
5 Flurouracil	1	30	30	32	30	32
5 Flurouracil	7	30	30	32	30	32
5 Flurouracil	14	25	35	30	25	30
5 Flurouracil	20	20	20	25	20	25
Vancomycin Antibiotic reference	-	25	28	29	N.A.	30
Cefodizime Antibiotic	-	N.A.	N.A.	N.A.	25	N.A.

of polymers binging the doxorubicin or chlorambucil was not observed release of tested drugs. It was not observed proliferation inhibition of cells MCF-7 and BALB/3T3 by doxorubicin as well as chlorambucil which were connected with the polymers in all time points (Table 3 and 5). The tendency to stimulate cells growth especially in case of BALB/3T3 cells was observed (Table 5). The obtained results are in agreement with previous works. [15]

The unexpected negative in vitro results of DOX and Chlorambucil released in culture media towards tested cell lines may be attributed to the effect of culture media on polymers loaded with both drugs or due to the blockage of pores in the synthesized polymers.

Antimicrobial Results:

The experiment was carried out in triplicate and the average zone of inhibition was calculated. The observed zones of inhibition are presented in Table 6.Antimicrobial activities were expressed as inhibition diameter zones in millimeters (mm). The experiment was carried out in triplicate and each average zone of inhibition was calculated.

Results revealed that the released 5-Fluorouracil from prepared polymer after 1d, 7d , 14 d and 20d gives the highest antimicrobial activity against all tested strains, followed by the released Doxorubicin and Chlorambucil.

Conclusion

1. The prepared nano-sized polymer was good and suitable carrier matrix holding drugs as controlled release drug system for anticancer.
2. In vitro study results indicated that the antiproliferative potency of the investigated SCACA/MSB polymer nanocomposites loaded with the anticancer drug 5-FU, DOX and Chlorambuciltowardbreast cancer cell line MCF-7 as well as normal cell line BALB/3T3 was around 80% at first days, followed by a slight decreased inhibition over the next 21days.
3. The antiproliferative effect of anticancer drugs released in water was directly proportional to the concentration, of drug.
4. The in vitro studies results of anticancer drugs released from the investigated polymer nanocomposites in culture media indicated that 5-FU is an effective and promising released from polymers compared to doxorubicin or Chlorambucil. The unexpected negative in vitro results of DOX and Chlorambucil released in culture media towards tested cell lines due to some blockage of pores in the synthesized polymers.
5. The antimicrobial examination revealed that the released 5 -Fluorouracil from prepared polymer after 1d, 7d , 14 d and 20d gave the highest antimicrobial activity against all tested strains, followed by the released Doxorubicin and Chlorambucil.
6. The prepared controlled release systems had multi effect as antiproliferative potency and antibacterial for some strains.

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