World Academy of Science, Engineering and Technology
International Journal of Pharmacological and Pharmaceutical Sciences
Vol:7, No:12, 2013

New Drug Delivery System for Cancer Therapy

Emma R. Arakelova, Stepan G. Grigoryan, Ashot M. Khachatryan, Karapet E. Avjyan, Lilia M. Savchenko, Flora G. Arsenyan

***Abstract*—**The paper presents a new drugs delivery system, based on the thin film technology. As a model antitumor drug, highly toxic doxorubicin is chosen. The system is based on the technology of obtaining zinc oxide composite of doxorubicin by deposition of nanosize ZnO films on the surface of doxorubicin coating on glass substrate using DC magnetron sputtering of zinc targets in $Ar:O_2$ medium at room temperature. For doxorubicin zinc oxide compositions in the form of coatings and gels with 180-200nm thick ZnO films, higher (by a factor 2) *in vivo* (ascitic Ehrlich's carcinoma) antitumor activity is observed at low doses of doxorubicin in comparison with that of the initial preparation at therapeutic doses. The vector character of the doxorubicin zinc oxide composite transport to tumor tissues ensures the increase in antitumor activity as well as decrease of toxicity in comparison with the initial drug.

***Keywords*—**Antitumor activity, doxorubicin, DC magnetron sputtering, zinc oxide.

I. Introduction

PRESENTLY many effective antitumor preparations are available however the problem of their target delivery causing minimum damage with minimal interaction with normal cells and other biological structures during their transport is put in the forefront.

Nanomedicine is dominated by nanoparticulate drug delivery systems because of their ability to cross biological barriers, accumulate at tumor sites and/or increase the solubility of drugs.

The use of targeted nanoparticles has made a significant contribution to diagnostics and therapy of cancerous diseases. Carbon nanotubes (CNTs) have been introduced recently as a novel carrier system for both small and large therapeutic molecules [1], [2]. The ability of CNTs to act as carriers for a wide range of therapeutic molecules, their large surface area and possibility to manipulate their surfaces and physical dimensions have been exploited for use in the photothermal destruction of cancer cells.

In [3], strong and selective uptake of boron nitride nanotubes by glioblastoma multiform cells but not by normal human fibroblasts was shown.

Recently, the use of ZnO quantum dots loaded with doxorubicin has proved to be an effective drug carrier characterized by an initial rapid drug release followed by a controlled release *in vitro* [4]. In this study, ZnO nanoparticles were encapsulated with chitosan to enhance the nanomaterial stability due to its hydrophilicity and cationic charge characteristics.

It is known that zinc oxide is not toxic and shows photocatalytic, antibacterial, antitumor activity under the influence of UV-radiation, while ZnO nanoparticles reveal such properties without UV irradiation [5]-[15].

These results confirm our earlier investigations [16]-[18], where it was shown that decomposition of hydrogen peroxide on the surface of zinc oxide target is accompanied by the formation of a Zn-containing complex, which increased by two orders of magnitude when the target surface was photoactivated using ultraviolet irradiation. When transforming to the gas phase, the complex decomposed on the surface of solid substrates with deposition of initial compound, ZnO. It is a chemical transport reaction (CTR), various modifications of which can receive wide acceptance in various areas of science and engineering. They are effective methods for transport of substances – just that determines their important role in both processes of formation of films, coatings and transport of drugs.

Our works [19]-[22] have demonstrated the modification of a series of amino acids using DC magnetron sputtering of ZnO target and thin zinc oxide film deposition on their surfaces in the form of coatings, composite films with polymers, by formation of their zinc oxide compositions. The effect of the size of modified surface, thickness of the nanosize ZnO film deposited on it, as well as compositions with polymers of various molecular weights ensuring prolonged action in animal organisms (mice), on antitumor activity and toxicity of the obtained compositions were studied. Effective antitumor zinc oxide compositions have been obtained revealing higher (by a factor of 2-2.5) antitumor activity and lower toxicity with respect to sarcoma 180 in comparison with initial antitumor compounds.

We are presenting a new drug delivery system based on the thin film technology for obtaining zinc oxide composite drugs, alternative to the traditional nanotubes, nanoparticles. ZnO nanosize films of a certain thickness are deposited directly on the drug surface in the form of coatings applied on glass substrate.

As a model antitumor drug, highly toxic doxorubicin is chosen.

Any system for delivery of antitumor preparations to tumor tissue includes scientific and engineering principles that comprise interdisciplinary (chemical, physical and biological) approaches.

Emma R. Arakelova, Ashot M. Khachatryan, Karapet E. Avjyan, and Lilia M. Savchenko are with State Engineering University of Armenia, 105 Teryan St., Yerevan 0009, Armenia (phone: (37410)565-136, fax: (37410)545-843; e-mail: emma_arakelova@yahoo.com).

Stepan G. Grigoryan and Flora G. Arsenyan are with Scientific Technological Center of Organic and Pharmaceutical Chemistry NAS RA, 26 Azatoutsyan ave, Yerevan 0014, Armenia (phone: (37410)522-021, fax: (37410)581-211; e-mail: grigstepan@yahoo.com).

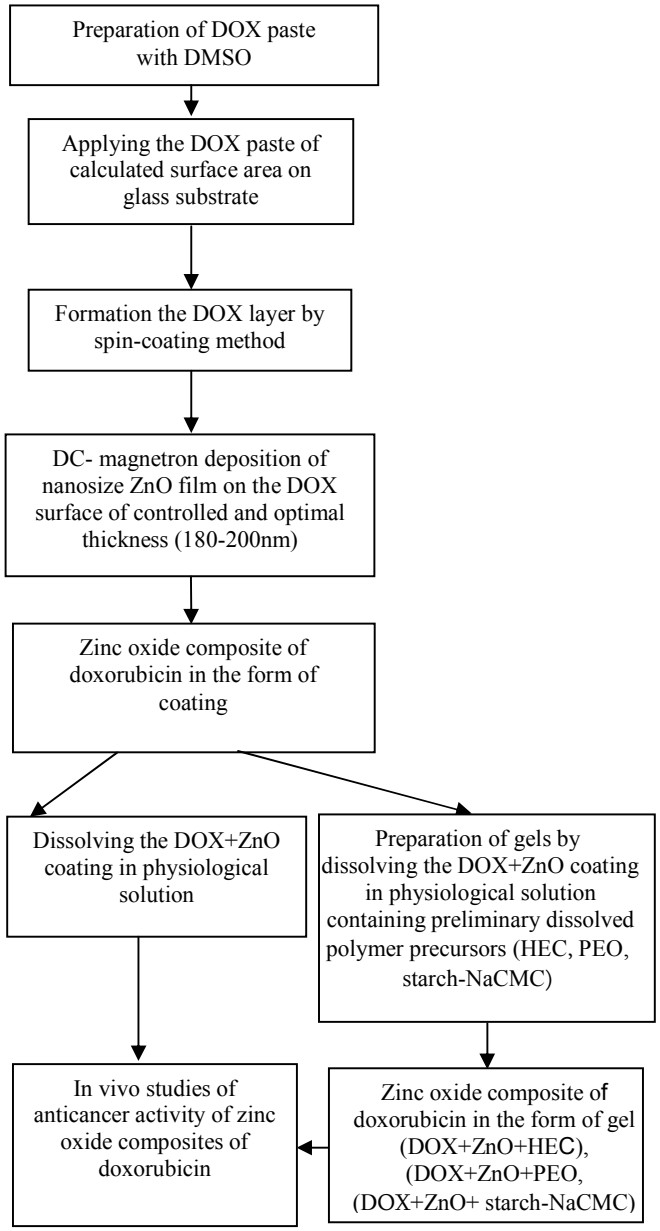


Fig. 1 Flow chart processing of zinc oxide composites of doxorubicin in the form of coatings and gels

Our delivery system is a model comprising various techniques (formation of coatings from doxorubicin, formation of hydrogels from doxorubicin, method of DC-magnetron deposition of ZnO thin films on surfaces of antitumor preparations, technique of identification of doxorubicin and its zinc oxide composites on strains of inoculate tumors) to obtain zinc oxide composites of antitumor preparations formed on the basis of a process model with optimum physical and chemical characteristics. To provide a combined description of the process and the structure of zinc oxide composites of antitumor preparations, it is necessary to describe several stages to develop a unified system model. First, for each of the offered techniques, its system model (accepted by the researches as resolvable) should be developed. Then all such models should be incorporated in the whole object model as a system, so a unified resolvable object model is formed as a system.

II. Materials and Methods

The paper is aimed at obtaining of zinc oxide compositions of widely used in chemotherapy antitumor drug, doxorubicin, which is characterized by its high anticancer activity and low toxicity.

To obtain coatings, gels from doxorubicin and its zinc oxide composites commercial doxorubicin preparation (lyophilized powder, "Belmedpreparaty" Company, Belorussia) is used.

A. Formation of Coatings from Doxorubicin

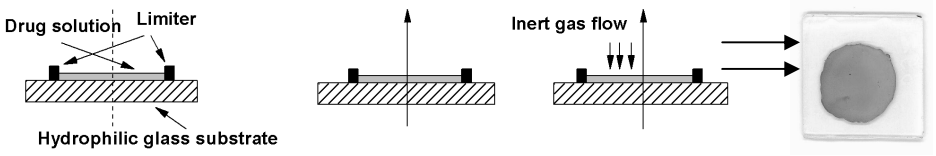
Coatings from doxorubicin (DOX) were formed with dimethyl sulfoxide (DMSO) on glass substrates of optimal surface areas presenting circles of a certain diameter depending on therapeutic doses for the animals for further obtaining of their zinc oxide composites. DMSO was used as a solvent to obtain doxorubicin in the form of pastes on glass substrates.

DOX coated substrates were placed in DC-magnetron for deposition of ZnO thin films on their surfaces.

The study was carried out with low doxorubicin doses in the zinc oxide composites taking into account the vector character of the doxorubicin zinc oxide composite transport to tumor tissue.

Coatings from doxorubicin at lower doses of doxorubicin (0.05 mg/mouse, 0.06 mg/mouse) than therapeutic one (0.1 mg/mouse) were formed on circular glass substrates for subsequent deposition of ZnO thin films (Fig. 2).

Fig. 2 shows the formation scheme of doxorubicin preparation in the form of coatings on hydrophilic glass substrates.



(a) No rotation (b) Slow speed rotation (c) High speed rotation (d) Doxorubicin on glass substrate

Fig. 2 The formation scheme of doxorubicin preparation in the form of coatings on hydrophilic glass substrates

The formation process of zinc oxide compositions of doxorubicin is related to their physical and chemical properties as well as sizes of surface areas, which determine Zn amount and thickness of ZnO nanosize films deposited on its surface.

The process of antitumor drug deposition by spin-coating method (Spin Coater VTC-100) was carried out according to the following principles:

- Determination of optimum deposition time for a given DMSO solution of medical preparation and a given deposition area.
- Determination of optimum velocity and time to obtain uniform coatings.
- Selection of neutral gases and determination of optimum rotation velocity for final drying of the obtained coatings.
- Control of the thickness coating uniformity.

B. Formation of Doxorubicin Hydrogels

At present polymeric gels and hydrogels are widely used in drug delivery systems due to their important physical and chemical properties such as controllable and prolonged release of drugs in organisms; as this takes place, high local concentration of medical preparations is maintained in the affected tissues over a long period [23], [24]. Injectable hydrogels contain polymer chains physically cross-linked by hydrogen bonds or van der Waals interaction or are in the form of linear polymer solutions subjected to reversible thermal gelation [25].

The polymeric gels: starch - sodium carboxymethyl cellulose (Starch+Na-CMC), 2-hydroxyethyl cellulose (HEC) and polyethylene oxide (PEO) in the system of target delivery of zinc oxide composites of doxorubicin were investigated.

Doxorubicin (DOX) with dimethyl sulfoxide (DMSO) in the form of paste was applied to glass substrate of a certain area depending on the selected dose of antitumor preparation. After drying at room temperature, the substrates with Dox coating were placed in DC magnetron to deposit nanosize zinc oxide films on their surfaces. The ZnO film thickness was determined by optimum technological parameters of its formation on the coating surface. The zinc oxide composite of doxorubicin obtained by deposition of ZnO thin film on the surface of glass substrates coated with doxorubicin (DOX+ZnO), was dissolved under ultrasonic (Ultrasonic processor CV 180) action (20 Hz, 60 sec, 65% amplitude) in physiological salt solution, where previously the following polymer was dissolved: starch - sodium carboxymethyl cellulose (Starch+Na-CMC, 1:1 weight %: 0.3%, 0.5%), 2-hydroxyethyl cellulose (HEC 0.3%), polyethylene oxide (PEO 0.3%).

C. DC-Magnetron Deposition of Nanosize ZnO Films on Surfaces of Doxorubicin

DC-magnetron deposition of thin ZnO films on the surface of antitumor preparations in the form of coatings was carried out using UVN-71-P3 modified device equipped with a measurement system and Ar, O_2 gases flow control regulators as well as Zn targets of certain geometry (Fig. 3).

The target geometry and high purity material (Zn of 99.99% purity) allowed the deposition of nanosize ZnO films on the surface of antitumor preparations at a vacuum of approximately 10^{-4} mm Hg to ensure high purity of the obtained zinc oxide compositions of antitumor preparations.

The measurement and control system (power and indication unit PR4000F) as well as the working gases control system (Ar and O_2 gases flow regulators MFC1179) ensured very accurate measurement and control (within the accuracy of ± 1 %) of the content, vacuum level in the chamber during the whole film formation process, at the same time providing stability of the process parameters and, hence, reproducibility of the properties of zinc oxide compositions to be obtained. When selecting technological regimes of nanosize ZnO film deposition (the working gases Ar: O_2 ratio, magnetron current, target-to-substrate distance and room substrate temperature) on the substrates (surfaces of antitumor preparations), it was necessary to take into account the conditions preventing undesirable side transformations of drugs.

Measurement and control of the thickness of ZnO films to be deposited was ensured by a device equipped with automatic control system ("Micron"). In off-line mode, an algorithm of deposition of ZnO films with the required thickness was used. ZnO nanosize films of a certain thickness were deposited on the surface of medical preparations with an accuracy of ± 0.1 nm

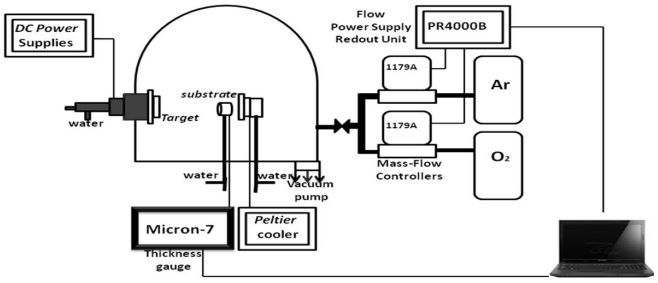


Fig. 3 Modernized DC magnetron sputtering device

D. Characterization of Doxorubicin and Zinc Oxide Composites

X-ray diffraction patterns were obtained on DRON-2.0 X-ray diffractometer.

FTIR spectra were recorded on NEXUS FT-IR spectrometer. UF spectra were recorded on Spectrofotometer TF-C-UVIS-SR 220-1100nm, Stellar net- Inc.com.

E. Method for Investigation of Antitumor Activity and Toxicity of Doxorubicin and Their Zinc Oxide Compositions

For the *in vivo* experiments, antitumor properties were studied on the model of mice transplantable tumor, ascitic Ehrlich's carcinoma (AEC), in accordance with the methodical recommendations on experimental research of antitumor substances.

The study was carried out in Toxicology and Chemotherapy Laboratory of Scientific Technological Centre of Organic and Pharmaceutical Chemistry of the National Academy of Sciences of the Republic of Armenia under conditions close to

GLP principles and European standards. The tumor transplantation was carried out by intraperitoneal introduction of ascitic liquid to the animals.

After the termination of the substance introduction, the animals were left to survive, and after their loss the antitumor effect was determined from the percentage of lifespan increase of animals against the control group.

III. Results and Discussion

Structural Characteristics and Antitumoral Activity of Zinc Oxide Composites of Doxorubicin in the Form of Coating and Gels

Optimum technological parameters of nanosize zinc oxide film by DC-magnetron deposition on the surface areas of doxorubicin in the form of coatings were determined.

The deposition of ZnO thin films on doxorubicin surface area was initiated for the purpose of direct delivery of drug to the tumour tissue.

Crystalline form of drugs ensures stability of their physical and chemical characteristics, which is important when carrying out investigations aimed at obtaining their optimum values.

Therefore, ZnO thin films deposition on glass substrates was investigated for the purpose of obtaining crystalline ZnO film for its further deposition on doxorubicin with a view to ensure doxorubicin zinc oxide composites.

X-ray investigation of nanosize ZnO films (DRON2.0 (λ CuKα) has shown that their structure depended on the power of the magnetron source. By the technique of magnetron sputtering of zinc target were produced amorphous samples of thin films of zinc oxide on glass substrates under pressures of approximately 0.001 mmHg, at the magnetron source power of 60 W, the working gases Ar:O_2 ratio of 3:1, and target–substrate distances in the range of 70–130 mm. In ZnO films deposited at the magnetron source power of 90 W, were observed polycrystalline structure. Raising the power of magnetron source from 90 to 120 W and increasing the target–substrate distances from 70 to 130 mm resulted in the transition from polycrystalline samples to ZnO films oriented along (002) crystallographic direction.

Optimum target-to-substrate distance, 13cm was selected taking into account the obtained dependences and uniformity of the ZnO films deposited on the surfaces of doxorubicin samples. At such target-to-substrate distance the film growth rate made 6.5-7 nm/min.

Composites on the basis of ZnO films and doxorubicin were obtained by deposition of nanosize zinc oxide film (using Zn targets) on doxorubicin deposited in the form of coatings in the following technological regime: operating current 400 mA, gas mixture Ar and O_2 at 70:30 ratio; operating pressure 10^{-4} mm Hg, target-to-substrate distance 13 cm.

IR spectra of doxorubicin (*lyophilized powder "Belmedpreparaty" Company, Belorussia*) and zinc oxide composites of doxorubicin (DOX+ZnO) were taken in nujol mull. Both spectra contain the following characteristic absorption bands: 3387, 3285cm^{-1} (valence vibrations of OH), 1720, 1616cm^{-1} (C=O), 1565cm^{-1} (aromatic ring), 1281,1264,1209,1082,1018cm^{-1} (deformation vibrations of OH), 701,631,584cm^{-1} (1,2,3 substituted aromatic ring). Comparison of DOX and DOX+ZnO absorption spectra has shown the presence of new bands of OH valence and deformation vibrations at 3325cm^{-1} and 1043cm^{-1}, correspondingly, as well as ZnO absorption band at 418cm^{-1}. Taking into account the obtained data, it is possible to come to the conclusion on interaction between ZnO and DOX, presumably, in the form of hydrogen bond.

UV-Vis spectra of doxorubicin and zinc oxide composites of doxorubicin were studied for various thicknesses of nanosize ZnO films deposited on the surface of doxorubicin coatings on glass substrates.

Fig. 4 presents reflection spectra of doxorubicin and doxorubicin zinc oxide compositions within the wavelength range of 235-1035nm. Table I presents some shifts of characteristic reflection bands of doxorubicin in doxorubicin zinc oxide composites depending on the time of deposition of nanosize ZnO films on the surfaces of the initial preparation. From Fig. 4 and Table I the changes of reflection spectra of doxorubicin zinc oxide composites at various times of deposition of nanosize ZnO films on doxorubicin coatings are obvious.

Red shift of doxorubicin reflection bands (342 and 510nm) was observed for doxorubicin zinc oxide compositions, as also evidenced by the change of DOX+ZnO coating color (toward red).

The shift of reflection peaks to long-wave region (up to 90nm) observed for doxorubicin zinc oxide compositions testified to the formation of intermolecular hydrogen bonds between doxorubicin and ZnO.

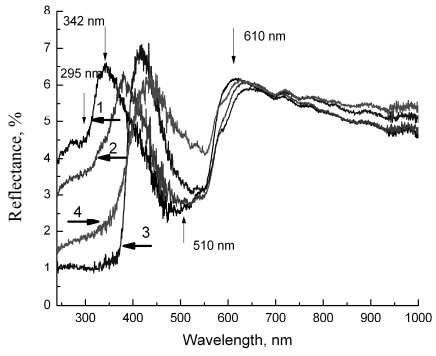


Fig. 4 Reflection spectra 1) DOX, 2) DOX+ZnO (t=8min), 3) DOX+ZnO (t=12min), 4) DOX+ZnO (t=28min)

As it is known from the publications, at UV irradiation or plasma treatment of ZnO films, electrons can interact with the Zn lattice ions forming Zn^+ defect regions [26] what is to our opinion favorable to the adsorption of the doxorubicin hydroxyl groups and leads to the formation of a charge transfer hydrophilic doxorubicin complex with ZnO. Plasma is a good source of ultraviolet irradiation, so plasma treatment

could be considered as an analogue of UV irradiation and the generation of electron-hole pairs on the ZnO – antitumor drug interface could promote the formation of charge transfer complexes. During the deposition and formation of nanosize zinc oxide films on the surface of doxorubicin, electron-hole pairs were apparently generated in plasma.

TABLE I
SHIFT OF CHARACTERISTIC REFLECTION BANDS OF DOXORUBICIN IN DOXORUBICIN ZINC OXIDE COMPOSITES DEPENDING ON THE TIME OF DEPOSITION OF NANOSIZE ZNO FILMS

Compound	Deposition time, min	ZnO film thickness on glass, min	Shift of reflection band of doxorubicin 342 and 510 nm	
DOX	-	-	342	510
DOX+ZnO	4	28	372	510
	6	42	382	510
	8	55	397	515
	12	86	407	525
	28	200	417	525
	33	235	432	544
	38	265	432	544

In zinc oxide composites of doxorubicin, control of ZnO film thickness, electron-hole pairs in plasma (nanosize ZnO film deposition period) and, thus the complex cation balance affects their antitumor activity and toxicity.

This fact apparently counts also in favor of the formation of the charge transfer hydrophilic doxorubicin complex.

Increase of antitumor activity and decrease of toxicity on the model of ascitic Ehrlich's carcinoma at lower doses of doxorubicin 0.05 mg/mouse, 0.06 mg/mouse than therapeutic one (0.1 mg/mouse) for coating and 0.06 mg/mouse for gels in the case of deposition of 180-200nm thick nanosize ZnO films was observed. Low antitumor activity was registered for doxorubicin zinc oxide composites in the case of deposition of 35-70nm thick ZnO films.

In the case of 250 thick nanosize ZnO films an excess amount of Zn resulted in a slight decrease of antitumor activity of the composites (Fig. 5, Table II).

The synergetic effect of ZnO and doxorubicin determines vector character of the transport of the doxorubicin zinc oxide compositions into the tumor tissue and results in the increase of antitumor activity in comparison with the initial preparation.

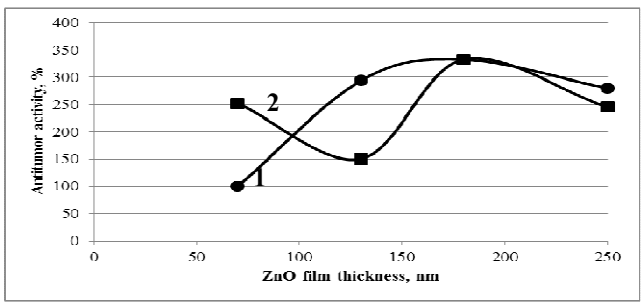


Fig. 5 Antitumor activity of zinc oxide composites of Doxorubicin vs. nano size ZnO film thickness: 1) in the coating form, 2) in the gel form

Thus, vector character of the transport of zinc oxide compositions to tumor tissues determines increase in their antitumor activity and decrease in toxicity, which is apparently related to the choice of optimum process parameters of DC-magnetron deposition, in particular, to the ZnO film thickness on the surface of antitumor drugs required to form a ZnO-drug complex.

TABLE II
ANTITUMOR ACTIVITY OF DOXORUBICIN, ITS ZINC OXIDE COMPOSITIONS DOX+ZNO AND POLYMER+DOX+ZNO WATER GEL SYSTEMS ON THE MODEL OF ASCITIC EHRLICH'S CARCINOMA

Composition	Daily dose (mg/mouse)	Deposition time of ZnO thin films, min	ZnO film thickness, nm	Antitumor action, %
DOX	0.1	-	-	170
DOX	0.05	-	-	150
DOX+ZnO (coating)	0.05	30	180	290
DOX	0.06	-	-	165
DOX+ZnO (coating)	0.06	10	70	100
		20	130	293.8
		30	180	332.3
		40	250	280
HEC+DOX	0.06	-	-	160
HEC+DOX+ZnO	0.06	10	70	251.9
		20	130	150.4
		30	180	332.6
		40	250	245
Strach+Na-CMC+DOX	0.06	-	-	169
Strach+Na-CMC+DOX +ZnO	0.06	30	180	384.1
PEO+DOX	0.06	-	-	175
PEO+DOX+ZnO	0.06	30	180	334.9

IV. CONCLUSIONS

A novel drug delivery platform based on zinc oxide composite drugs formed by DC magnetron deposition of nanosize ZnO films of a certain thickness on the surface of drug coating is developed.

By the example of doxorubicin zinc oxide composites in the form of coatings and gels, higher (by a factor 2) *in vivo* antitumor activity was observed on the model of ascitic Ehrlich's carcinoma for 180-200nm thick ZnO films in comparison with that of doxorubicin at much lower doses than therapeutic dose.

Such system of vector delivery has several basic advantages: purity of zinc oxide compositions formed in vacuum, chemical simplicity, target delivery of the composites to tumor tissue due to formation of a charge transfer complex (accepted assumption), formation of hydrogen bond between doxorubicin and ZnO (confirmed by IR, UV spectroscopy as well as X-ray diffraction methods).

The specified method stands out favorably by its wide opportunities of controlling the process of formation of the compositions at room deposition temperature, when there is no undesirable side transformations, simplified techniques of

controlling thickness of ZnO films deposited on the surface of antitumor preparations, layer-by-layer deposition of films, which allows synthesis of the composites with predetermined technological characteristics.

ACKNOWLEDGMENT

This work was supported by the ISTC #A-1962 project.

REFERENCES

[1] M. A. Elhissi, W. Ahmed, A. Israr Ul Hassan, R. Dhanak Vinod., and A. D'Emanuele, "Carbon nanotubes in cancer therapy and drug delivery," Journal of Drug Delivery, pp. 1-10, 2012.

[2] Z. Liu, K. Chen, C. Davis, S. Sherlock, Q. Cao, X. Chen, and H. Dai1, "Drug delivery with carbon nanotubes for in vivo cancer treatment," *Cancer Res*., vol. 68 (16), pp. 6652–6660, 2008.

[3] G. Ciofani**,** V. Raffa**,** A. Menciassi, and A. Cuschieri, "Folate functionalized boron nitride nanotubes and their selective uptake by glioblastoma multiforme cells: implications for their use as boron carriers in linical boron neutron capture therapy," *Nanoscale Res. Lett*., pp.113-121, 2008.

[4] Q. Yuan, S. Hein, R. D. Misra, "New generation of chitosan-encapsulated ZnO quantum dots loaded with drug: Synthesis, characterization and in vitro drug delivery response," *Acta Biomater*, pp. 2732–2739, 2010.

[5] L. Jingyuan, G. Dadong Xuemei, H. Wang, H. Jiang Wang, B. Chen, "The photodynamic effect of different size zno nanoparticles on cancer cell proliferation in vitro," *Nanoscale Res. Lett*., vol 5, pp. 1063-1071, 2010.

[6] K. Yogendra, K. M. Mahadevan, N. Sunee, N. Madhusudhana. "Photocatalytic activity of synthetic ZnO composite against Coralene red F3BS dye in presence of solar light," *Int. J. Environ. Sci*., vol. 1(5), pp. 839-846, 2011.

[7] K. Ghule, A. Vithal Ghule, B.-J. Chen, and Y.-C. Ling, "Preparation and characterization of ZnO nanoparticles coated paper and its antibacterial activity study," *Green Chem.***,** vol 8, pp. 1034 – 1041, 2006.

[8] C. Hanley, J. Layne, A. Punnoose, K. M. Reddy, I. Coombs, A. Coombs, K. Feris, and D. Wingett, "Preferential killing of cancer cells and activated human T cells using ZnO nanoparticles," *Nanotechnology*, vol. 19, pp.1-10, 2008.

[9] S. Ostrovsky, G. Kazimirsky, A. Gedanken, and C. Brodie., "Selective cytotoxic effect of ZnO nanoparticles on glioma cells," *Nano Res*., vol 2, pp. 882- 890, 2009.

[10] D. Guo, C. Wu, H. Jiang, Q. Li, X. Wang, and B. Chen, "Synergistic cytotoxic effect of different sized ZnO nanoparticles and daunorubicin against leukemia cancer cells under UV irradiation," *J Photochem. Photobiol. B,* vol 93, pp. 119–126, 2008.

[11] S. Nair, A. Sasidharan, V. V. Divya Rani, D. Menon, S. Nair, K. Manzoor, and S. Raina, "Role of size scale of ZnO nanoparticles and microparticles on toxicity toward bacteria and osteoblast cancer cells," *J Mater Sci Mater Med*. vol 20, pp. 235–241, 2009.

[12] C. Hanley, A. Thurber, C. Hanna, A. Punnoose, J. Zhang, and D. G. Wingett, "The influences of cell type and ZnO nanoparticle size and immune cell cytotoxicity and cytokine induction," *Nanoscale Res Lett*. vol 4, pp. 1409–1420, 2009.

[13] H. Wang, D. Wingett, M.H. Engelhard, K. Feris, K. M. Reddy, P. Turner, J. Layne, C. Hanley, J. Bell, D. Tenne, C. Wang, and A. Punnoose, "Fluorescent dye encapsulated ZnO particles with cellspecific toxicity for potential use in biomedical applications." *J Mater. Sci. Mater. Med*., vol 20, pp. 11–22, 2009.

[14] W. J. Rasmussen, E. Martinez, L. Panagiota, and D. G. Wingett, "Zinc oxide nanoparticles for selective destruction of tumor cells and potential for drug delivery applications," *NIH PA*., vol 7(9), pp. 1063-1067, 2010.

[15] B. Wang, W. Feng, M. Wang, T. Wang, Y. Gu, M. Zhu, H. Ouyang, J. Shi, F. Zhang, Y. Zhao, Z. Chai, H. Wang, and J. Wang, "Acute toxicological impact of nano- and submicro-scaled zinc oxide powder on healthy adult mice," *J. Nanopart. Res.,* vol 10, pp. 263–276, 2008.

[16] E. Arakelova, V. Parvanyan, F. Grigoryan, G. Asatryan, and G. Petrosyan, "Increase in concentration of a Zn-containing volatile complex by UV irradiation of a target for ZnO films synthesis," EPD Congress 2006, TMS (The Minerals, Metals & Materials Society), San Antonio, Texas, pp. 813-818, 2006.

[17] E. Arakelova, F. Grigoryan V. Parvanyan, and G. Asatryan, "Study of decomposition regularities for a Zn-containing volatile complex used in ZnO films synthesis," EPD Congress 2006, TMS (The Minerals, Metals & Materials Society), San Antonio, Texas, pp. 883-888, 2006.

[18] V. G. Parvanyan, F. A. Grigoryan, E. R. Arakelova, G. G. Asatryan, G. N. Mirzoyan, and G. G. Petrosyan. "Formation and decomposition regularities of a Zn-containing volatile complex for ZnO films synthesis," Kinetika i kataliz, v.48, (3), pp. 1-4, 2007.

[19] E. Arakelova, A. Khachatryan, K. Avjyan, A. Voskanyan, Z. Farmazyan, A. Mirzoyan, T. Jivanshiryan, L. Savshenko, S. Ghazaryan, K. Grigoryan, and F. Arsenyan, "Zinc oxide nanocomposites with antitumor activity," *Nat. Sci.*, vol 2(2), pp. 1341-1348, 2010.

[20] S. H. Minasyan, S. H. Ghazaryan, V. S. Tonoyan, S. A. Bajinyan, K. P. Grigoryan, F. T. Greenaway, and J. R. S. Sorensen, "Synthesis, characterization and measurement of antioxidant reactivity of Salicylidene-DLTyrosine ethyl esters and copper(II) Salicylidene-DL-Tyrosine ethyl esters)$_2$ in linoleic acid peroxidation reaction system," *Synth. React. Inorg. Met.-Org. Nano-Metal Chem*., vol 36(5), pp. 425-434, 2006.

[21] E. Arakelova, A. Khachatryan, K. Avjyan, Z. Farmazyan, L. Savshenko, A. Mirzoyan, S. Ghazaryan, and F. Arsenyan, "Method of obtaining anticancer composite films and coatings". Patent Republic of Armenia, № 2481A, 25.01.2011.

[22] A. Mirzoyan, E. Arakelova, and Z. Farmazyan, "Antitumor zinc oxide composites of salicyliden amino acids," *Vestnik (Russian)*, vol 79(2), pp. 492-499, 2012.

[23] R. T. Hoare and S. D. Kohane. "Hydrogels in drug delivery: Progress and challenges," *Polymer,* vol 49, pp.1993-2007, 2008.

[24] C. Sutton, "Adhesions and their prevention," *The Obstetrician and Gynecologist*, vol 7, pp. 168-76, 2005.

[25] L. Yu, Z. Zhang, H. Zhang, and J. Ding, "Biodegradability and biocompatibility of thermoreversible hydrogels formed from mixing a sol and a precipitate of block copolymers in water," *Biomacromolecules*, vol. 11, pp. 2169–78, 2010.

[26] D. P. Subedi, D. K. Madhup, A. Sharma, U. M. Joshi, and A. Huczko, "Study of the wettability of ZnO nano films," *Int. Nano Lett*., vol 1(2), pp. 117-122, 2011.