Vietnam Journal of Science and Technology ***57*** *(3B) (2019) 1-8*
doi:10.15625/2525-2518/57/3B/14044

EFFECT OF FISH SCALE COLLAGEN ON SOME CHARACTERISTICS AND DRUG RELEASE OF CARRAGEENAN/COLLAGEN/ALLOPURINOL FILM

Tran Thi Mai[1], Nguyen Thuy Chinh[1, *], Vu Quoc Manh[2, 4], Nguyen Thi Thu Trang[1], Tran Do Mai Trang[1], Vu Quoc Trung[3], Ha Van Hang[5], Thai Hoang[1, 2]

[1]*Institute for Tropical Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay District, Ha Noi*

[2]*Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay District, Ha Noi*

[3]*Faculty of Chemistry, Hanoi National University of Education, No. 136 Xuan Thuy Road, Cau Giay District, Ha Noi*

[4]*Faculty of Foundation Science, College of Printing Industry, Phuc Dien Road, Bac Tu Liem District, Ha Noi*

[5]*Ministry of Public Security Institute of Science and Technology, 47, Pham Van Dong, Mai Dich, Cau Giay District, Ha Noi*

[*]Email: *ntchinh@itt.vast.vn*

Received: 29 July 2019; Accepted for publication: 1 November 2019

Abstract. Collagen from fish is attracting a lot of attention thanks to its high absorbance ability, biocompatibility as well as non-religious obstruction and cheap sources. It could be applied in many fields, for example: food, cosmetic, or biomedicine. Using of collagen also helps reduce the environment pollution from fish scale waste in fish processing. In this study, collagen extracted from Vietnamese fresh-water tilapia fish scales was used in combination with carrageenan for the improvement of drug release control. The influence of fish scale collagen content on morphology, thermal behavior and drug release from carrageenan/collagen/ allopurinol composite film was evaluated by methods such as field emission scanning electron microscopy (FESEM), differential scanning calorimetry (DSC) and ultraviolet-visible spectroscopy (UV-Vis). From the DSC data, FESEM analysis and drug release of carrageenan/collagen/allopurinol composite films, the most suitable collagen in composite film is 5 wt.%.

Keywords: fish scale collagen, drug release, carrageenan and biomedicine.

Classification numbers: 2.7.1, 2.9.3, 2.9.4.

1. INTRODUCTION

Collagen is a type of protein found in animals, especially mammals. It is among the most

abundant fibrous proteins used in many fields due to its many advantages such as good tensile strength [1-2], biodegradable, non-toxic [3] and high biological compatibility [4]. It is considered to be one of the best biological materials to use in medicine when compared to other natural polymers [5]. In sources of collagen extraction, collagen from fish is currently being studied as a substitute for collagen extracted from terrestrial animal skins [6-8]. Collagen extracted from fish scales is a type I collagen. Nowadays, collagen from fish is attracting a lot of attention thanks to its high absorbance ability, biocompatibility, non-religious obstruction and cheap sources. Collagen extracted from fish scales could be applied in many fields, for example, food, cosmetic, or biomedicine. Use of collagen also helps reducing the environment pollution from fish scale waste in fish processing. In our previous studies, collagen has been successfully extracted from fish scale in northern Viet Nam [9].

In recent years, bio-polymers have been increasingly studied by many researchers all over the world since they are abundant in nature, renewable, biocompatible and cost – effective. Among them, carrageenan, a water-soluble fiber, is found in many seaweeds such as red and brown algae. It is widely used in the food, cosmetic and pharmaceutical industries with functions such as gelling, thickening and stabilizing, etc. [10]. Combination of carrageenan with other biopolymer, for example collagen, could form a new material which has the ability to control drug release. Based on our previous studies, it can be reported that the poly(lactic acid)/chitosan, chitosan/alginate composite films containing different drugs prepared by the solution method have good drug release control ability [11-12].

Allopurinol is known as a xanthine oxidase inhibitor and a medication used to treat gout or kidney stones, and to decrease high blood uric acid levels. The drug half-life is 1-2 hours, and it has a short half-life so it is suitable for sustained release drug delivery [13]. In this paper, allopurinol will be used as a model drug loaded by carrageenan/collagen films.

From the literature reviewed, the effect of collagen content extracted from fish scales on the characteristics of carrageenan/collagen composite films carried allopurinol drug was investigated and presented in this paper. Their characteristics consist of morphology, thermal properties, and drug release were measured and analyzed by FESEM, DSC, and UV-Vis methods.

2. EXPERIMENTAL

2.1. Materials

The carrageenan used was of white powder with pH between 7.5 to 10.5 and humidity ≤ 12 %, and the allopurinol was of white powder with purity ≥ 98 %, melting temperature ~ 300 - 350 ^{o}C. Both of them were purchased from SigmaAldrich (USA). Collagen was extracted from tilapia scales in Northern provinces of Viet Nam with purity > 99 %. Calcium chloride ($CaCl_2$, 94 %), sodium hydroxide (NaOH), ethyl alcohol (ethanol, 99.7 %), methyl alcohol (methanol, 99.7 %), and acetic acid (CH_3COOH) were commercial products of China.

2.2. Synthesis of carrageenan/collagen/allopurinol film composites

The process of preparing carrageenan/collagen/allopurinol composites is as follows: Carrageenan was dissolved in distilled water at a temperature of 80 ^{o}C for 30 minutes, then it was cooled to about 40-50 ^{o}C and $CaCl_2$ solution was added to the above solution. The solution was then stirred for 30 minutes at room temperature (denoted as solution A). Collagen and allopurinol were dissolved in CH_3COOH 1 M solution (solution B) and NaOH 0.5 M solution

was prepared (solution C). Solution C was added slowly into solution A at the rate of 2 ml/min while the solution A was being stirred on magnetic stirrer (step 1). After that, solution B was added slowly into the mixture of solutions A and C at the rate of 3 ml/min while the mixture was being stirred on magnetic stirrer, same as step 1 (step 2). Then, the solution mixture at step 2 was stirred for 2 hours to stabilize. Finally, the solution mixture was poured into glass plates and allowed to evaporate naturally to obtain a carrageenan/collagen/allopurinol composite film. The weight and content of the components in the composite films are presented in Table 1.

Table 1. Weight, content of components and the symbols of carrageenan/collagen/allopurinol composite films.

No.	Symbol	Carrageenan		Collagen		Allopurinol	
		Mass (g)	Content (wt.%)	Mass (g)	Content (wt.%)	Mass (g)	Content (wt.%)
1	CC95-5	0.095	95	0.005	5	-	-
2	ACC99-1	0.099	99	0.001	1	0.005	5
3	ACC97-3	0.097	97	0.003	3	0.005	5
4	ACC95-5	0.095	95	0.005	5	0.005	5
5	ACC93-7	0.093	93	0.007	7	0.005	5
6	ACC90-10	0.090	90	0.010	10	0.005	5

2.3. Characterizations

Field emissions canning electron microscope (FESEM): FESEM images of collagen and carrageenan/collagen/allopurinol composite films were conducted on a S-4800 instrument (Hitachi, Japan) at the Institute of Materials Science, Vietnam Academy of Science and Technology.

Differential scanning calorimetry (DSC): DSC diagrams of carrageenan/collagen/allopurinol composite films were recorded on a DSC131 thermal analyzer (Setaram, France) at the Department of Chemistry – Hanoi University of Science. The samples were measured in the temperature range from room temperature to 400 ^{o}C with heating speed of 10 ^{o}C/min and in a nitrogen gas environment.

2.4. *In-vitro* drug release test

Drug release of allopurinol from composite films was carried out as follows: samples (drug or drug-loaded by carrageenan/collagen films) were taken in vessels containing certain amount of release media and the drug release was assessed according to testing time. This method is most popular for *in vitro* release testing of polymers carrying drugs [14-15]. In this study, the certain amount of samples is 0.015 g which was put into 200 ml of buffer solution (pH 2 and pH 7.4 solutions, corresponding to the pH in the stomach and duodenum in the human body) at 37 ^{o}C ± 0.5 ^{o}C. The testing was carried out continuously for 32 hours with a rotation speed of 400 rpm. After 1 testing hour, 5 ml of sample solution was drawn out and filtered through a hydrophilic membrance.The same volume of fresh dissolution buffer was added to replace the

withdrawal amount after each sampling. The amount of drug released was determined by UV-visible spectrophotometer method. After that, the drug amount of allopurinol released from the composite was calculated with a standard curve prepared using bracketed concentration of allopurinol in each pH = 2 buffer solution and pH = 7.4 buffer solution. The standard curves of allopurinol in pH = 2 and pH 7.4 buffer solutions are y = 10382x + 0.051 (λ_{max} = 257.49 nm, linear regression coefficient R^2 = 0.9997) and y = 1898x + 0.0047 (λ_{max} = 249.81 nm, R^2 = 0.998), respectively. All the tests were carried out at the Institute for Tropical Technology, Vietnam Academy of Science and Technology.

3. RESULTS AND DISCUSSION

3.1. Thermal properties of carrageenan/collagen/allopurinol composite films

Figure 1 shows the DSC diagram of carrageenan/collagene/allopurinol composite films. The characteristics of composites films like melting temperature and melting enthalpy are presented in Table 2. It can be seen that all three component samples (like as ACC99-1, ACC95-5 and ACC90-10) have endothermic peak around at around 70 ^oC. This endothermic peak corresponds to the modified temperature of collagen. This result is consistent with the result of determining pure collagen (73.0 ^oC). However, this endothermic peak does not appear in the CC95-5 composite film (without allopurinol). This may be due to collagen having good interaction with carrageenan. From the Table 2, the ACC99-1 sample (containing 1wt.% of collagen) has the highest modified temperature (73.5 ^oC), and the modified temperature of this sample gradually goes down to 61.2 ^oC when increasing the collagen content to 5 wt.%. This value of composite film containing 10 wt.% of collagen (ACC90-10 sample) is 66.4 ^oC. Among investigated samples, the composite film containing 5 wt.% of collagen (ACC95-5 sample) has lowest modified temperature. It suggests that collagen has strongly interacted with carrageenan at 5 wt.% of collagen.

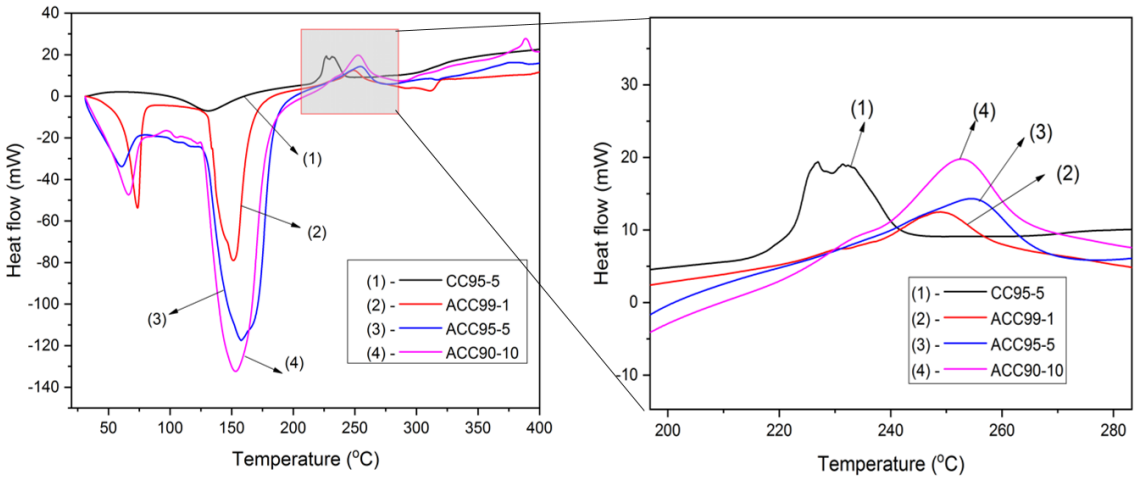

Figure 1. DSC diagram of composite films (CC95-5 (1), ACC99-1 (2), ACC95-5 (3) and ACC90-10 (4)).

The Figure 1 also indicates that all of samples have 2 other peaks, which are endothermic peak and exothermic peaks at around 150 ^oC and 250 ^oC, respectively. The endothermic peak and exothermic of composite films are correspondingly representing for the melting and

decomposing temperature of collagen and carrageenan. For examples, the melting and decomposing temperature of CC95-5 sample are 131.3 ^{o}C and 226.9 ^{o}C, respectively. The melting temperature of composite film is fluctuated along with the change of collagen contents. It is 151.4, 157.9 and 153.6 ^{o}C for the composite film containing 1 wt.%, 5 wt.% and 10 wt.% of collagen, respectively.

Another issue is that the temperature of composite films containing allopurinol (ACC samples) being larger than that of the composite film without allopurinol (CC95-5 sample). This is explained by the melting temperature of allopurinol being higher than that of collagen and carrageenan, so the melting temperature of the composite film is increased when mixing allopurinol with collagen and carrageenan. This also indicates that these components of composite films can be interacted well together. Besides, the increase in melting temperature leads to rising up of melting enthalpy (Table 2). It is clear that the CC95-5 composite film has smallest melting temperature and melting enthalpy is of 464.58 J/g. By contrary, the melting enthalpies of the drug carrying composite films (ACC samples) is higher than that of CC95-5. Among the drug carrying composite films, the composite film containing 5 wt. % of collagen (ACC95-5) has smallest value of melting enthalpy (599.82 J/g). Thus, the ACC95-5 composite film has the lowest crystallinity due to the melting enthalpy is proportional to crystallinity. The degradation temperature of composite films containing allopurinol tends to rise up compared with that of the composite film without allopurinol, for example, the CC95-5 sample has the lowest degradation temperature (226.9 ^{o}C) while the ACC991-1, ACC95-5 and ACC90-10 samples have degradation temperature at around 250 ^{o}C (Figure 1b). The degradation enthalpy of composite films is higher than that of composite film without allopurinol, for example, the CC95-5 and the ACC95-5 composite films has highest value (-45.07 J/g).

Table 2. DSC data of carrageenan/collagen/allopurinol composite films, T_m: melting temperature, T_{dm}: maximum degradation temperature, ΔH_m: melting enthalpy, ΔH_d: degradation enthalpy.

Samples	T_{m1} (^{o}C)	T_{m2} (^{o}C)	T_{dm} (^{o}C)	ΔH_m (J/g)	ΔH_d (J/g)
CC95-5	-	131.3	226.9	464.58	-156.15
ACC99-1	73.5	151.4	248.3	760.51	-82.87
ACC95-5	61.2	157.9	254.1	599.82	-45.07
ACC90-10	66.4	153.7	252.3	858.82	-90.65

From the above results, it can be seen that the composite film containing 5 % of collagen has lowest crystallinity degree. This may increase the ability to release allopurinol drug from carrageenan/collagen/allopurinol composites films.

3.2. Morphology

FESEM images of carrageenan/collagen and carrageenan/collagen/allopurinol composites at the same magnification are presented in Figure 2.

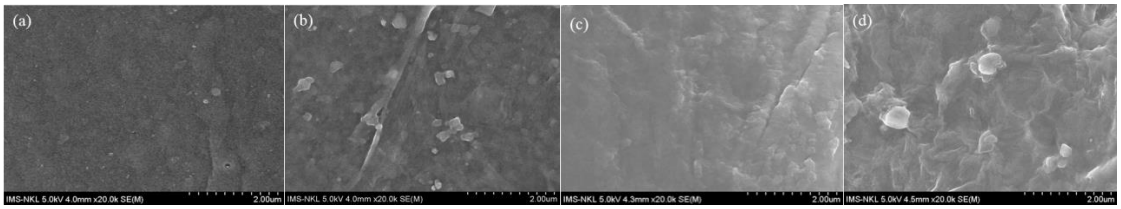

Figure 2. FESEM image of carrageenan/collagen/allopurinol composite films with: (a) CC95-5, (b) ACC99-1, (c) ACC95-5 and (d) ACC90-10.

It can be easily seen that the carrageenan/collagen composites (CC95-5) composite film has a relatively homogeneous structure due to good interaction between the carrageenan and collagen (Figure 2a). When the allopurinol is added into the carrageenan/collagen blends, the structure of composite films becomes separated two-phase structure with dispersed phase (allopurinol) in polymers (carrageenan and collagen) (Figures 2b, c and d). From Figure 2c, the composite containing 5 wt.% of collagen (ACC95-5) has a more uniform structure compared with others composites with the different collagen content because the separation of phases is not clear in this samples and difficult to observe. Therefore, the dispersion of allopurinol into polymers is best at 5 wt.% of collagen and this is consistent with the above results.

3.3. Drug release

The drug content of allopurinol released from composite films (ACC99-1, ACC97-3, ACC95-5, ACC93-7, ACC90-10) in buffer solutions is displayed in Figure 3. It can be seen that, the release of allopurinol from composite films occured in two stages: (1) the rapid release process took place for first testing 11 hours, and (2) then, the slow one indicated the controlled release of drugs at the end of period (the next testing 17 hours).

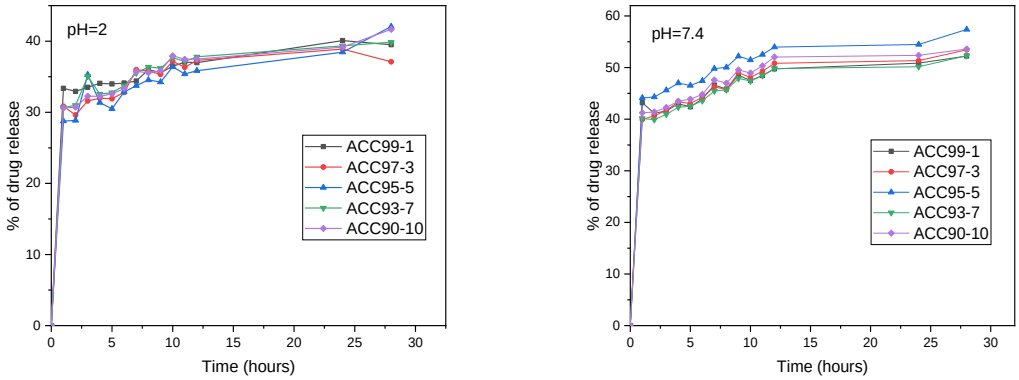

Figure 3. In vitro drug release of allopurinol from carrageenan/collagen/allopurinol composite films in pH = 2 and pH = 7.4 buffer solutions.

Figure 3 also demonstrates that the ratio of carrageenan and collagen significantly affected the release of allopurinol from carrageenan/collagen/allopurinol composite films in buffer solution pH = 2. The content of allopurinol released from ACC955-5 (carrageenan/collagen/ allopurinol ratio of 95/5/5, wt.%) is the smallest in all samples at the rapid release stage (period

1), but it is highest (42.06 %) at the period 2 (the controlled release stage) after testing 28 hours. This may be due to the fact that the interaction between collagen and carrageenan in this ratio is stronger than that of composite films with other ratios of collagen and carrageenan, so the crystallinity of ACC95-5 is reduced as mentioned above. The amount of allopurinol released from the ACC95-5 composite film is more than that from other samples during allopurinol released testing in buffer solution pH = 2.

In the buffer solution pH = 7.4, among the investigated composite films, the ACC95-5 composite film has the highest amount of allopurinol released. For example, the allopurinol amount released for testing 12 hours and testing 28 hours are 53.96 and 57.39 %, respectively. The drug release content from ACC99-1, ACC97-3, ACC95-5, ACC93-7, ACC90-10 composite films in pH = 7.4 solution are 52.23 %, 53.43 %, 57.39 %, 52.29 % and 53.60 % after testing 28 hours, respectively. This indicates that the most suitable content of collagen for best released drug is 5 % which corresponding to the sample having a 95:5 ratio of carrageenan and collagen (ACC95-5). The allopurinol amount released from the composite film in buffer solution pH = 7.4 is significantly higher than that released in buffer solution pH = 2. The rapid release of allopurinol is occurred may be due to the drug on the surface of compositefilm being released. On the other hand, the interactions between collagen and carrageenan are hydrogen bonding and bipolar interaction, so the diffusion ability of allopurinol into solution is increased.

4. CONCLUSSION

In this work, the carrageenan/collagen composite films containing allopurinol is prepared by solution method. The results of DSC analysis indicate that the composite film containing 5 % of collagen (ACC95-5) has the highest melting temperature and the smallest melting enthalpy. This proves that the ACC95-5 composite film is the most durable. FESEM images show that the carrageenan/collagen composites (CC95-5) composite film has homogeneous structure while the carrageenan/collagen/allopurinol composite films have structure with two separate phases (a matrix phase and dispersed phase). The composite film containing 5 % of collagen (ACC95-5) has a more uniform structure compared with other composites having different collagen content. The result of released drug indicates that the ratio of carrageenan and collagen significantly influences on the release of allopurinol from carrageenan/collagen/allopurinol composite films. The content of allopurinol released from ACC955-5 composite film is highest (42.06 %) in both buffer solutions (pH 2 and 7.4). The release ability of allopurinol in buffer solutions is arranged in the following order: pH 7.4 > pH 2.

Acknowledgements. The authors would like to thank the National Foundation for Science and Technology Development in Vietnam for financial support (subject code 104.02-2017.326, period of 2018–2021).

REFERENCES

1. Nishikawa A. K., Taira T., Yoshizato K. - In vitro maturation of collagen fibrils modulates spreading, DNA synthesis, and collagenolysis of epidermal cells and fibroblasts, Exp Cell Res. **171** (1987) 164–177.
2. De Vries H. J. C., Middelkoop E., Mekkes J. R., Dutrieux R. P., Wildevuur C. H. R., Westerhof W. - Dermal regeneration in native noncross-linked collagen sponges with diferent extracellular matrix molecules, Wound Repair Regen. **2** (1994) 37–47.

3. Lin Y. C., Tan F. J., Marra K. G., Jan S. S., Liu D. C. - Synthesis and characterization of collagen/hyaluronan/chitosan composite sponges for potential biomedical applications, Acta Biomater. **5** (2009) 2591–2600.
4. Nehrer S., Breinan H. A., Ramappa A., Young G., Shortkroff S., Louie L. K., Sledge C. B., Yannas I. V., Spector M. - Matrix collagen type and pore size influence behaviour of seeded canine chondrocytes, Biomaterials **18** (1997) 769–776.
5. Naresh K., Shahin S. A., Vikash K. D., Utpal B. - Exploiting the potential of collagen as a natural biomaterial in drug delivery, Journal of Proteins and Proteomics **1** (1) (2010) 9-14.
6. Prashant K. B., Padma B. D. - Isolation, characterization and valorizable applications of fish scale collagen in food and agriculture industries, Biocatalysis and Agricultural Biotechnology **7** (2016) 234-240.
7. Sijin C., Hui C. - Rapid isolation of high purity pepsin-soluble type I collagen from scales of red drum fish (*Sciaenopsocellatus*), Food Hydrocolloids **52** (2016) 468-477.
8. Huong L.T.T., Dzung N. H., Tuan P. D. – Extraction and purification of collagen from the skins of basa fish (*Pangasiushypophthalmus*), Vietnam Journal of Science and Technology **52** (4) (2014) 431-440.
9. Chinh N. T, Manh V. Q., Trung V. Q., Trang T. D. M., Hoang T. - Extraction of hydroxyapatite and collagen from the Vietnamese tilapia scales, Vietnam J. Chem. **57** (2) (2019) 225-228.
10. Liang L., Rui N., Yang S., Shirui M. - Carrageenan and its applications in drug delivery, Carbohydrate Polymes **103** (2014) 1–11.
11. Trang N. T. T., Chinh N. T., Giang N. V., Thanh D. T. M, Lam T. D., Hoang T. - PLA/CS/Nifedipinenanocomposite films: Properties and the in-vitro release of nifedipine, Journal of Electronic Materials **45** (7) (2016) 3581–3590.
12. Chinh N. T., Ly N. T. H., Mai T. T., Trang N. T. T., Loc T. T., Giang L. D., Tung N. Q., Hoang T. - Characteristics and properties of chitosan/alginate polymer blend carrying lovastatin drug, Vietnam Journal of Science and Technology **54** (2B) (2016) 118-124.
13. Pacher P., Nivorozhkin A., Szabó C. - Therapeutic effects of xanthine oxidase inhibitors: renaissance half a century after the discovery of allopurinol, Pharmacological Reviews **58** (1) (2006) 87–114.
14. Xu X., Khan M. A., Burgess D. J. - A two-stage reverse dialysis in vitro dissolution testing method for passive targeted liposomes, Int J. Pharm. **426** (2012) 211–218.
15. Asadishad B., Vossoughi M., Alamzadeh I. - In vitro release behavior and cytotoxicity of doxorubicin-loaded gold nanoparticles in cancerous cells, Biotechnol. Lett. **32** (2010) 649–654.