International Journal of Drug Delivery 2 (2010) 278-286
http://www.arjournals.org/ijdd.html
Research article
ISSN: 0975-0215

Novel thermosensitive poly (N-isopropylacrylamide-co-vinylpyrrolidone-co-methacrylic acid) nanosystems for delivery of natural products

Omid Mashinchian[1, 3], Roya Salehi[2], Gholamreza Dehghan[3], Ayoub Aganejad[1], Soudabeh Davaran[2, 4]*, Yadollah Omidi[1, 5]*

***Corresponding author:**
Yadollah Omidi
[1]Research Center for Pharmaceutical Nanotechnology, Tabriz University of Medical Sciences, Tabriz, Iran
[2]Drug Applied Research Center, Tabriz University of Medical Sciences, Iran
[3]Departments of Plant Sciences, Faculty of Natural Sciences, University of Tabriz, Iran
[4]Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran
[5]School of Advanced Biomedical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran
Email: yomidi@tbzmed.ac.ir
davaran@tbzmed.ac.ir

Abstract

The purpose of this research was to synthesize polymer based smart nanosystems for delivery of important bioactive natural products such as sesquiterpene coumarin derivatives of *ferula szowitsiana, farnesiferol C* as a potent anticancer. To this aim, polymeric micelles were prepared using *N*-isopropylacrylamide (NIPAAM), vinyl pyrrolidone (VP) and methacrylate (MAA) as monomers which were cross-linked with *N*, *N*-methylene *bis*-acrylamide (MBA). The molar ratio of the PNIPAAm: VP: MAA group was 75.7:9.5:14.8. These micelles were further characterized upon their physicochemical properties using particle size analyzer, FT-IR, H-/C-NMR, HPLC. Particle size analyzer resulted in ~500 nm micelles with ~95% drug entrapments. Drug release from the polymeric micelles after 300 hours at 37°C and 40°C were 60 and 98 %, respectively. Upon these findings, it is proposed that the P (N-isopropylacrylamide-co-Methacrylic acid-co-Vinylpyrrolidone) micelles may be considered as thermosensitive delivery nanosystem.

Keywords: N-isopropylacrylamide; Thermosensitive; Nanoparticles; Farnesiferol C; Amphiphilic polymers.

Introduction

Herbal medicine has been regarded as primitive medicaments paradigms for many years. These kinds of remedies are believed to possess great curing potentials for a wide range of diseases as they appear to be safer medicines possessing an inveterate precedence [1, 2]. It seems that the emergence of the phytomedicines with smart delivery systems (e.g., synthetic/biodegradable polymer based nanosystems) may advance development of more efficient herbal medicines. So far, many natural products have been reported with different therapeutic effects.

Of these, based upon our preliminary findings, the species of *Ferula* (subfamily of *Umbelliferae*) grown in the Mediterranean area such as northwest of Iran [3] produces secondary metabolites with anti-cancer activities. Likewise, some recently published works have reported this genus as a good source of biologically active compounds such as sesquiterpene and coumarin derivatives (e.g., *Farnesiferol C*) [3]. Interestingly, the isolated compounds from this genus were shown to possess different therapeutic effects in cancers and leishmaniosis [3, 4].

doi:10.5138/ijdd.2010.0975.0215

Further, polymeric micelles structured from amphiphilic copolymers are regarded as one of the most promising carriers for drug delivery [5, 6, 7]. The self-assembled structures are composed of two basic compartments of hydrophobic core and hydrophilic corona, at which they have been used to solubilize poorly soluble drugs/compounds [8, 9, 10]. Owing to such supramolecular structure, the hydrophobic drugs can associate with the inner cores of polymers, while the hydrophilic shells of polymer interact with the external environment. These polymeric self-assembled have been considered as one of the promising drug carriers which confer higher drug concentrations in an aqueous milieu where the solubility is deemed to limit the partitioning of hydrophobic drugs [11, 12, 13, 14, 15, 16]. Poly (N-isopropylacrylamide) (PNIPAAm) and related copolymers have been widely investigated as stimuli sensitive polymers [17, 18]. PNIPAAm polymers display unique reversible thermosensitivity with the hydrated extended coil to globule transition by increasing temperature over its lower critical solution temperature [17, 18, 19, 20].

In this study, we report the synthesis and characterization of the PNIPAAm nanostructured polymers, which were used as a smart thermosensitive nanocarrier for delivery of *farnesiferol C.*

Materials and methods

Chemicals preparations

NIPAAm (Fluka, Deisenhofen, Germany) was purified by recrystallization in hexane and dried under vacuum at 25°C. VP (Merck, Hohenbrunn, Germany) was freed from stabilizer by twice vacuum distillation with continuous bubbling argon. Methacrylic acid (MAA) (Fluka, Deisenhofen, Germany) was used as supplied. Initiator ammonium persulfate (APS) (Sigma-Aldrich Co., Steinheim, Germany) was purified by recrystallization in EtOH:H2O (2:1) solvent mixture and dried under vacuum at 40°C and N, *N*%-Methylene *bis*-acrylamide (MBA) (Sigma-Aldrich Co., Steinheim, Germany) was used directly without further purification. Sodium monohydrogen phosphate and dihydrogen phosphate was purchased from Sigma-Aldrich Co. (Steinheim, Germany). Silica gel (60F 254) and acetone were product of Merck (Darmstadt, Germany).

Preparation of Extract and Chromatography

The roots of *F. szovitsiana* were collected from Khoy (West Azerbaijan province, north-west of Iran). They were air-dried at room temperature and powdered. The chloroform extract was prepared by macerating the powder for 48 h with three changes of solution at room temperature. The combined solvent extracts were evaporated to yield a brownish, viscous residue (8% yields). The residue was fractionated by thin layer chromatography (TLC) on silica gel using petroleum ether: ethyl acetate (2:1) as the solvent system. The fractions were visualized under UV light at 254 nm and then eluted using acetone. Active constituent was abundantly purified as follow: briefly, 15 g the extract was subjected to column chromatography on silica gel (5_50 cm) using petroleum ether with increasing volumes of acetone [petroleum ether(100), petroleum ether–acetone (95 : 5), (90 : 10), (85 : 15), (80 : 20), (75 : 25), (70 : 30), (60 : 40), (50 : 50) and acetone (100)]. The fractions were compared by TLC (silica gel using petroleum ether–EtOAc as solvent), and those giving similar coumarin spots were combined and further purified on preparative TLC to give *Farnesiferol C* (5.43 mg). The melting point, H- and C-NMR data of the obtained isolated *farnesifeol C* were confirmed according to previously published works [21, 22, 23].

Synthesis of Farnesiferol C loaded nanopolymers

A copolymer of NIPAAM-VP-MAA was synthesized through free radical mechanism. Water soluble monomers, NIPAAM, VP and MAA, were used in 75.7:9.5:14.8 molar ratios and the polymer were cross-linked with MBA. Using a standard experimental protocol, 900 mg NIPAAM, 98 mg VP and 38 ml MAA (also freshly distilled) were mixed in 50 ml of water. To cross-link the polymer chain, 300 ml of MBA (0.049 g/ml) was added in the aqueous solution of monomers. Predetermined amount *of Farnesiferol-C* was included to reaction solution. The dissolved oxygen was removed by passing nitrogen gas for 30 min. To initiate the polymerization, APS (0.3 mol% with respect to the monomers) was added to mixture. The polymerization was fulfilled at 30°C for 24 h in nitrogen atmosphere. The mixture was purified by dialysis for 5 days using dialysis membrane (Cellu SepH1) with MWCO of 2000 and the external aqueous solution was removed two times

a day and displaced with fresh distilled water. The dialyzed aqueous solution of polymeric micelles was ultracentrifugation (8000 rev.min^{-1}) immediately to obtain dry powder for subsequent use. The yield of micelle nanoparticles was above 80%.

Determination of drug content of the nanoparticles

To determine drug entrapment within the nanoparticles, 100 mg dried powder of nanoparticles was dissolved in 100 mL water. After complete dissolution of the polymer, the designated amount of drug was quantified using a high performance liquid chromatography (HPLC) method. The HPLC system consisted of a Waters 515 pump, automatic injector (7plus Waters autosampler), and Waters 2487 dual k absorbance detector. Chromatographic separation was achieved with a Nucleosil (Phenomenex) ODS 5 lm (25 cm 3 4.6 mm, i.d.) column and HPLC grade water, acetonitrile (15/85) with 0.1% trifluoroacetic acid (TFA) as the mobile phase at a flow rate of HPLC flow rate 1 mL.min^{-1} (in a stepwise increasing up to 2 mL.min^{-1}) and the pressure was adjusted up to 1100 psi. The UV detection was at 320 nm. The run time for the assay was 15 min and the retention time for Farnesiferol-C was 7.349 min. *Farnesiferol-C* loading efficiencies of nanoparticles were measured using following equation [18, 11]:

Loading efficiency = (A/B) ×100

Where, A and B are the amount of remained drug in the polymer and initial feeding amounts of drug, respectively.

Drug release studies

The in vitro release of *Farnesiferol C* from nanoparticles was carried out at 37 and 40°C. *Farnesiferol C* suspension (2mL containing nanoparticles 100 mg) was taken into a dialysis membrane (cut off: 2000; Sigma-Aldrich Co., Steinheim, Germany). It was allowed to float in a beaker containing 20 mL of phosphate buffered saline (PBS; pH 7.4). The beakers were placed in a shaker incubator maintained at 37°C and 40°C in 100 rev/min. Aliquots of 2 mL were removed from the external buffer solution on designated time point up to 300 hours and the lost volume was replenished with fresh PBS. The released *Farnesiferol C* was analyzed using a HPLC assay as described previously. The results were presented in the terms of cumulative release as a function of time:

$$\text{Cumulative amount released}(\%) = \left(\sum_{t=0}^{t=t} M_t / M_o \right) \times 100$$

Where, $\sum_{t=0}^{t=t} M_t / M_0$ is the cumulative amount of released *Farnesiferol C* from the nanoparticles at time t, and M_0 is the total amount of *Farnesiferol C* in the nanoparticles [24, 18, 17].

Physicochemical characterization of polymeric micelles and Farnesiferol C

FT- IR spectra of polymer and *Farnesiferol C* were taken in KBr pellet using Shimadzu Fourier Transformed Infrared (FT-IR) spectrophotometer instrument (Shimadzu FT-IR 8400 S, Kyoto, Japan).

The chemical composition of the synthesized *Farnesiferol C* was determined by ^{1}H NMR and ^{13}C-NMR (Bruker spectra spin 400 MHz, Leipzig, Germany).

The average size of the polymeric nanoparticles was observed by using a laser diffraction particle size analyzer (Shimadzu SALD 2101, Kyoto, Japan).

Results and discussion

Synthesis and characterization of NIPAAM-VP-MAA copolymeric micelles

Random copolymerization of NIPAAM with VP and MAA, i.e. poly (NIPAAM-VP-MAA), was accomplished by radical polymerization process of the micellar aggregates of the monomers. These polymers possess amphiphilic characteristic, presumably with a hydrophobic core inside the micelles and hydrophilic outer shell composed of hydrated amides, pyrrolidone and carboxylic groups projected from the monomeric units. Water insoluble drug like *Farnesiferol C* was dissolved into the hydrophobic core of the polymeric micelles. Figure 1 shows the FT-IR spectra of poly (NIPAAM-VP-MAA), where an intense peak at 3345 cm^{-1} can be attributed to the COOH group of MAA. The C-H stretching vibration of the polymer backbone is manifested through strong peak at 2928 cm^{-1}. Absorbance of amide carbonyl groups in PNIPAAm occurs at 1650 cm^{-1}, bending frequency of amide N–H appears at 1550 cm^{-1}.

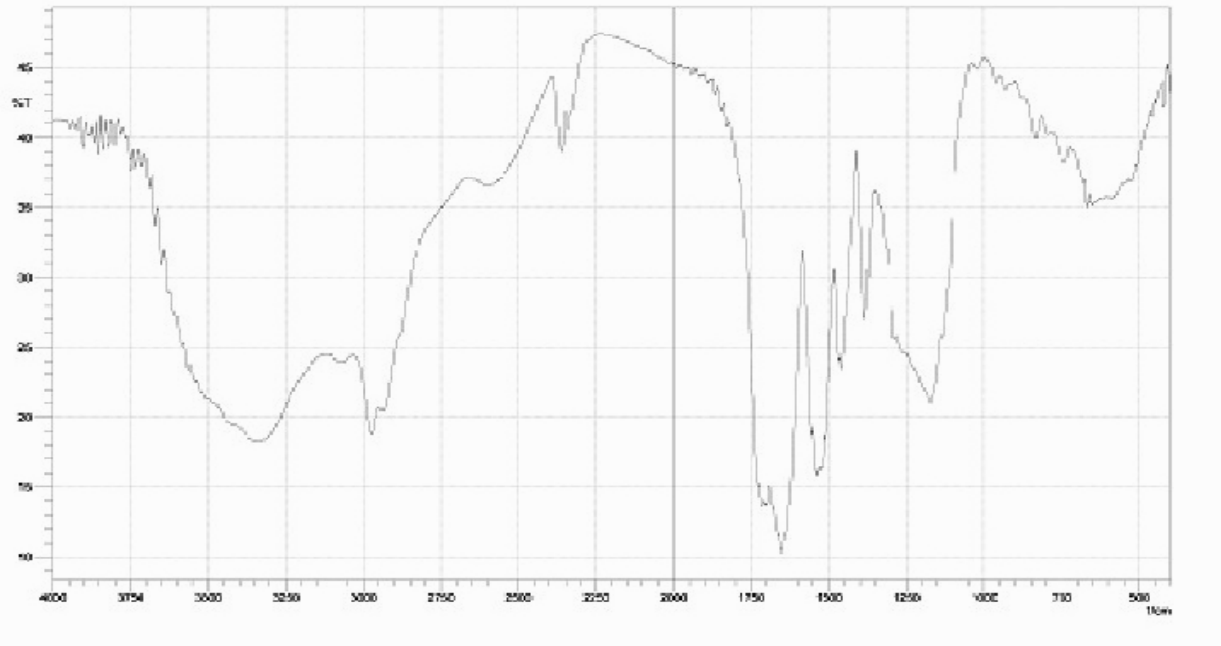

Figure 1. The FT-IR spectrum of NIPAAM-VP-MAA copolymeric micelles.

Characterization of Farnesiferol C: ^{1}H-NMR, ^{13}C-NMR of Farnesiferol C

Figure 2 represents the molecular structure of Farnesiferol *C*. This coumarin based compound displays substantial structural commonalities in ^{1}H-NMR (Figure 3) and ^{13}C-NMR (Figure 4) spectrums.

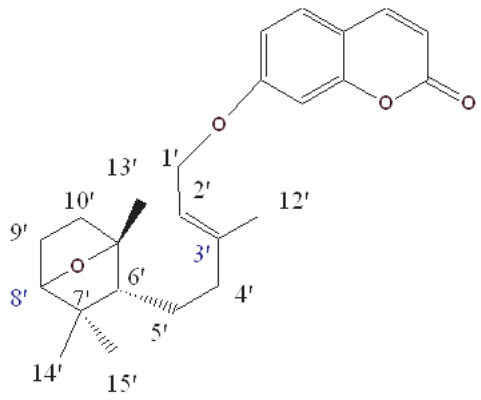

Figure 2. Structure of *Farnesiferol C.*

As shown in Figure 3, the most important characteristic of ^{1}H-NMR is the presence of two doublet peaks at 6.2 and 7.6 ppm that related to 3, 4 protons and two doublet peaks at 6.8 and 7.3 ppm related to 5, 6 protons in coumarin ring, respectively. One doublet peak at 3.72 ppm related to 8' proton, one doublet peak at 4.6 ppm related to 1', one triplet peak at 5.45 ppm related to 2', two singlet peaks at 1.33 and 1.76 ppm related to 12' and 13' protons respectively and two singlet peaks at 1.02 and 1.03 ppm related to 14' and 15' protons respectively (Table 1). As shown in Figure 4, the ^{13}C-NMR spectrum reveals two short peaks at 161 and 162 ppm respectively related to 7 and 2 carbons. Also, a peak at 65-70 ppm is related to 11' carbon. This distinct peak displays a constant characteristic of the coumarin structure. These data are in consensus with the results reported previously [21, 3].

***In vitro* drug release**

One of the main goal in this research is investigation of stimuli responsive, especially temperature sensitive polymeric nanoparticles an efficient drug carrier. To investigate the effect of environmental temperatures on drug release behaviors, In vitro *Farnesiferol C* release from the micelles was studied in PBS at 37 °C and 40°C. As illustrated in Figures 5, the *Farnesiferol*

C loaded nanoparticles showed well-developed sustained drug release patterns which was in correlation with temperature. Polymeric micelles yielded the initial burst release of *Farnesiferol C*, reaching 24 and 50 % in first two days at 37 °C and 40°C, respectively. The initial burst release of *Farnesiferol C* from nanoparticles is much faster at higher temperatures.

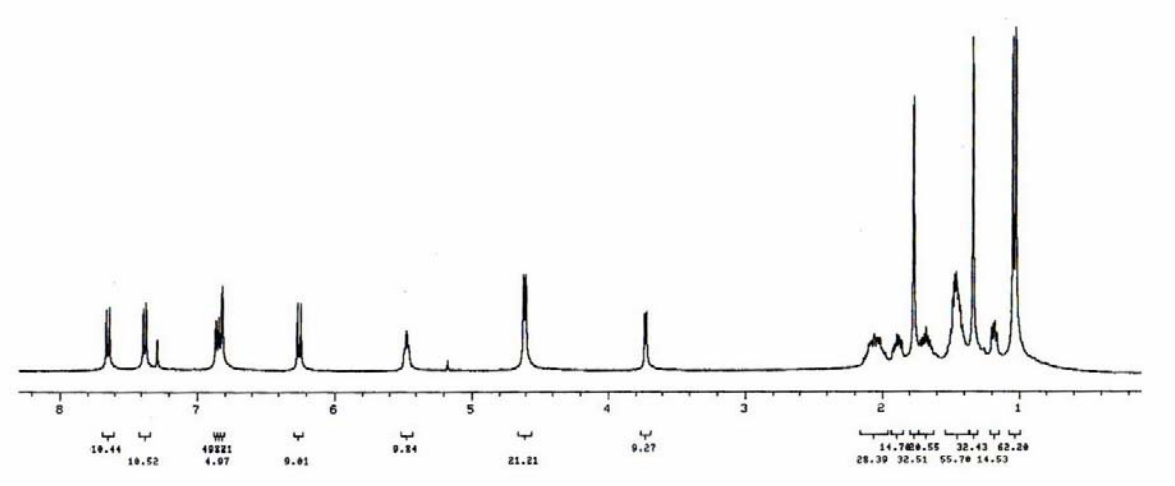

Figure 3. The ^{1}H-NMR spectrum of *Farnesiferol C.*

When the temperature increased, the outer shell collapsed. After 300 hours incubation at 37°C and 40°C, the drug release from micelles was respectively 60% and 98%. Such low release is attributed with the insolubility of the drug in aqueous solution as well as the low diffusion coefficient of *Farnesiferol C* in the micelle core. The collapsed shell might induce the deformation of the core–shell structure, exposing *Farnesiferol C* molecules to the external in vitro release medium and thus accelerating drug release.

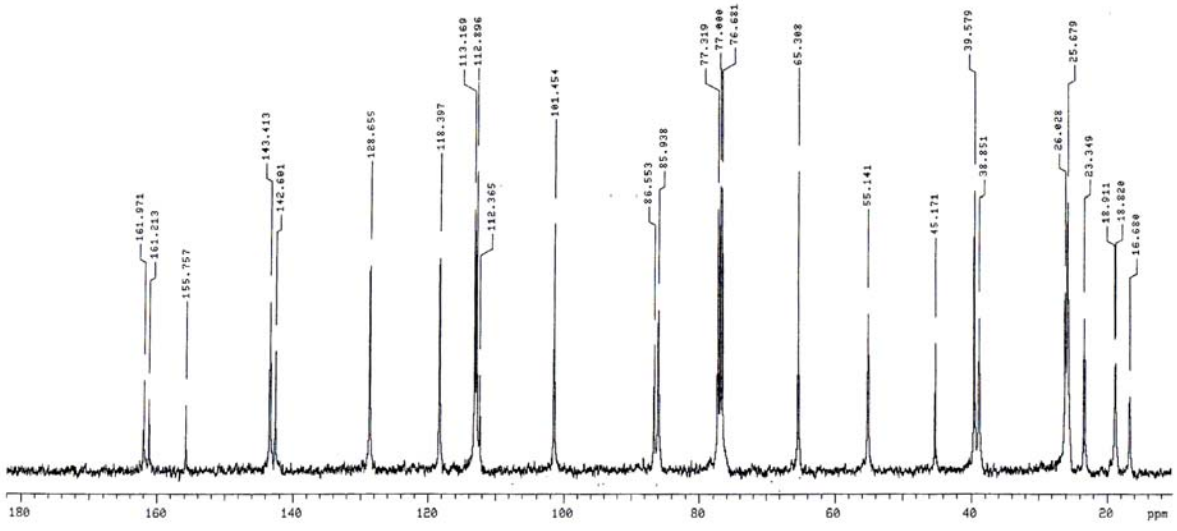

Figure. 4. The ^{13}C-NMR spectrum of *Farnesiferol*.

Solubilities and other physicochemical properties of nanoparticles are essential requirements for drug advancement from pharmaceutical technology towards clinical trials and applications [25, 26, 27]. Given the integration of nanomaterials with herbal medicine for providing more efficient medications, we focused on exploitation of bioactive derivatives from plant resources that provide huge potential for novel biologically active compounds such as anti-inflammatory, anticancer, antiviral, antibacterial, and antioxidant agents. *Ferula* genus from *Umbelliferae* family, which is naturally distributed throughout the Mediterranean to Central Asia [21, 22, 3], seems to confer interesting bioactivities.

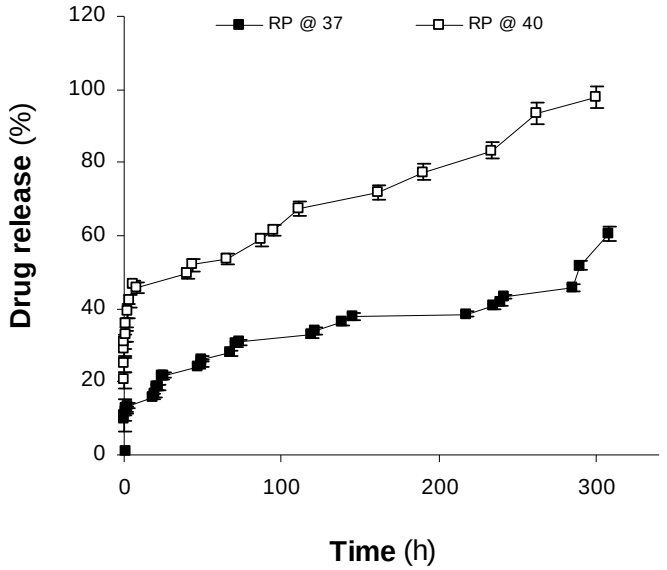

Figure 5. In vitro *Farnesiferol C* release from NIPAAM-VP-MAA copolymeric micelles as a function of time at pH 7.4 in 37 ºC and 40 ºC.

In our preliminary investigation, we have found some non-polar fractions from a chloroform extract of *ferula szowitsiana* roots, which were examined for its anticancer activities using several human cancer cell lines by means of the routinely used MTT assay (our unpublished data). Similarly, Dehghan et al., (2007), recruiting acridine orange/Ethidium bromide double staining methodology, reported the induction of caspase–3 activities leading to apoptosis in the ht-29 cells treated with *Farnesiferol C*. intriguingly, the morphological studies showed chromatin condensation and detaching of adherent cells treated with chloroform extract and fractions of *Farnesiferol C*, resulting in profound inhibition of the cell proliferation in human cancer cell lines perhaps through induction of apoptosis [28, 21, 22, 4]. The relatively high cytotoxic potential *Farnesiferol C* directed us to formulate it as a nano-scaled product using biodegradable polymers to maximize its biological impact. In fact, it is now well documented that exploitation of appropriate nanomaterials can improve the effectiveness of treatment, perhaps though more efficient targeting potential of these nano-structured medicines in cancer cells while imposing minimal adverse consequences [25]. Of these, the amphiphilic block copolymers have been intensively used as nano-/micro-micelles for drug delivery and targeting purposes. In fact, the selfaggregates formed by polymeric amphiphiles are composed of hydrophobic core and hydrophilic corona. Owing to such supramolecular structure, they have been considered as a promising drug delivery system. It has been reported to confer higher drug concentration in an aqueous milieu by partitioning the drugs into the hydrophobic core compartment, in particular when the solubility issues limit accessibility of the hydrophobic drugs. These characteristics can grant an excellent physiological stabilities and biocompatibilities in the blood stream to achieve long-circulating time [29]. This long-circulating ability in turn makes it possible to accumulate the encapsulated drugs in the required regions via an active and/or a passive mechanism, in particular when they are used for targeting of cancer [6, 7, 5].

Upon our data, the poly (NIPAAm-MAA-VP) nanoparticles showed no chemical interaction with the model drug and we witnessed fairly high capacity of loading efficiencies (95-99%) for *Farnesiferol C* which is a hydrophobic compound. To investigate the effect of temperatures on drug release pattern, the release experiments were performed at two different temperatures of 37 °C and 40 °C. The effect of PNIPAAm hydrogel layers on the nanoparticle surface at higher temperature affected drug release patterns (at 40°C). Owing to the increase of hydrophobicity of the poly (NIPAAm-MAA-VP) nanoparticles shell compartments, the sustained drug

release patterns were increased. Therefore, it is possible to conclude that the formation of PNIPAAm hydrogel layer at higher temperatures might be act as additional diffusion barrier for the drug release.
The initial burst release of *Farnesiferol C* was attributed to *Farnesiferol C* molecules located within the corona or at the interface between the micelle core and corona [30, 31].

Table 1. H-NMR information of *Farnesiferol C* produced from *ferula szowitsiana.*

Number of proton	δ ppm(J)
3	6.25 d
4	7.65 d
5	7.37 d
6	6.84 d
8	6.82 d
1'	4.6 d
2'	5.45 t
4',5'	1.85, 2.09 m
8'	3.72 d
12'	1.33 s
13'	1.76 s
14',15'	1.02 s,1.04s

This clearly implies that this thermally responsive amphiphilic nanopolymer would be able to be used for the encapsulation of poorly soluble hydrophobic drugs. Also the *Farnesiferol C* loaded nanoparticles were stable for more than one month at physiological conditions. Therefore we can use this system as a good carrier to sustained drug release at physiological environments. Another main foci on this research is investigation of stimuli responsive, especially temperature sensitive polymeric nanoparticles an efficient drug carrier.

Conclusion

To achieve a stimuli–response targeting mechanism, we aimed to synthesize and characterize the poly (NIPAAm-MAA-VP) as a thermosensitive polymeric nano-scaled micelle for hydrophobic drugs, in which the *Farnesiferol C* was opted as an anticancer agent. Owing to the amphiphilic charactereistics of these nanostructures, the poly (NIPAAM-VP-MAA) formed self-assembled nanoparticles which displayed fairly high potentials to be used as drug carriers. The release profile showed faster release rate of *Farnesiferol C* from *Farnesiferol C* -loaded micelles at 40°C, which clearly indicates thermosensitive potential of these nanosystems. Upon these findings, we propose that these micelle might provide opportunities to construct smart drug delivery systems.

References

1. Iranshahi M, Arfa P, Ramezani M, Jaafari MR, Sadeghian H, Bassarello C, Piacente S, Pizza C. Sesquiterpene coumarins from Ferula szowitsiana and in vitro antileishmanial activity of 7-prenyloxycoumarins against promastigotes., 2007; 68:554-561.
2. Reiner Z, Tedeschi-Reiner E. The effects of plant sterols on hypercholesterolemia. Lijecnicki Vjesnik, 2007; 129:276-281.
3. Iranshahi M, Kalategi F, Rezaee R, Shahverdi AR, Ito C, Furukawa H, Tokuda H, Itoigawa M. Cancer chemopreventive activity of terpenoid coumarins from Ferula species. Planta Medica, 2008; 74:147-150.
4. Soga O, Van Nostrum CF, Fens M, Rijcken CJF, Schiffelers RM, Storm G, Hennink WE. Thermosensitive and biodegradable polymeric micelles for paclitaxel delivery. J Controll Rel, 2005; 103:341-353.
5. Cheng C, Wei H, Shi BX, Cheng H, Li C, Gu ZW, Cheng SX, Zhang XZ, Zhuo RX. Biotinylated thermoresponsive micelle self-assembled from double-hydrophilic block copolymer for drug delivery and tumor target. Biomaterials, 2008; 29:497-505.
6. Wei H, Zhang X, Cheng C, Cheng SX, Zhuo RX. Self-assembled, thermosensitive micelles of a star block copolymer based on PMMA and PNIPAAm for controlled drug delivery. Biomaterials, 2007; 28:99-107.
7. Chung JE, Yamato M, Yokoyama M, Aoyagi T, Sakurai Y, Okano T. Thermo-responsive drug

delivery of polymeric micelles incorporating adriamycin. Proceedings of the Controlled Release Society, 1998; 380-381.
8. Calvo P, nchez A, nez J, pez MI, Calonge M, Pastor JC, Alonso MJ. Polyester nanocapsules as new topical ocular delivery systems for cyclosporin A. Pharma Res, 1996; 13:311-315.
9. Calvo P, Vila-Jato JL, Alonso MJ. Comparative in vitro evaluation of several colloidal systems, nanoparticles, nanocapsules, and nanoemulsions, as ocular drug carriers. J Pharma Sci, 1996; 85:530-536.
10. Choi C, Chae SY, Nah JW. Thermosensitive poly(N-isopropylacrylamide)-b-poly(e-caprolacto ne) nanoparticles for efficient drug delivery system. Polymer, 2006; 47:4571-4580.
11. Hu Y, Zhang L, Cao Y, Ge H, Jiang X, Yang C. Degradation behavior of poly(epsilon-caprolactone)-b-poly(ethylene glycol)-b-poly(epsilon-caprolactone) micelles in aqueous solution. Biomacromol, 2004; 5:1756-1762.
12. Kim SY, Lee YM, Kang JS. Indomethacin-loaded methoxy poly(ethylene glycol)/poly(D,L-lactide) amphiphilic diblock copolymeric nanospheres: Pharmacokinetic and toxicity studies in rodents. J Biomed Mater Res - Part A, 2005; 74:581-590.
13. Mainardes RM, Evangelista RC. PLGA nanoparticles containing praziquantel: Effect of formulation variables on size distribution. Int J Pharm, 2005; 290:137-144.
14. Panyam J, William D, Dash A, Leslie-Pelecky D, Labhasetwar V. Solid-state solubility influences encapsulation and release of hydrophobic drugs from PLGA/PLA nanoparticles. J Pharm Sci, 2004; 93:1804-1814.
15. Shenoy D, Little S, Langer R, Amiji M. Poly(ethylene oxide)-modified poly(?-Amino Ester) nanoparticles as a pH-sensitive system for tumor-targeted delivery of hydrophobic drugs: Part 2. In vivo distribution and tumor localization studies. Pharma Res, 2005; 22:2107-2114.
16. Salehi R, Davaran R, Rashidi HR, Entezami A. Thermosensitive nanoparticles prepared from poly(N-isopropylacrylamide-acrylamide-vinilpyr rolidone) and its blend with poly(lactide-co-glycolide) for efficient drug delivery system. J App Poly Sci, 2009; 111:1905-1910.
17. Salehi R, Arsalani N, Davaran S, Entezami AA. Synthesis and characterization of thermosensitive and pH-sensitive poly (N-isopropylacrylamide-acrylamide-vinylpyrrolidone) for use in controlled release of naltrexone. J Biomed Mater Res Part A, 2008; 9999:NA.
18. Neradovic D, Soga O, Van Nostrum CF, Hennink WE. The effect of the processing and formulation parameters on the size of nanoparticles based on block copolymers of poly(ethylene glycol) and poly(N-isopropylacrylamide) with and without hydrolytically sensitive groups. Biomaterials, 2004; 25:2409-2418.
19. Uchida K, Sakai K, Ito E, Hyeong Kwon O, Kikuchi A, Yamato M, Okano T. Temperature-dependent modulation of blood platelet movement and morphology on poly(N-isopropylacrylamide)-grafted surfaces. Biomaterials, 2000; 21:923-929.
20. Dehghan G, Shafiee A, Ghahremani MH, Ardestani SK, Abdollahi M. Antioxidant potential of various extracts from Ferula szovitsiana in relation to their phenolic content. Pharm Biol, 2007; 45:691-699.
21. Dehghan G, Solaimanian R, Shahverdi AR, Amin G, Abdollahi M, Shafiee A. Chemical composition and antimicrobial activity of essential oil of Ferula szovitsiana D.C. Flavour and Fragrance Journal, 2007; 22:224-227.
22. Shahverdi AR, Fakhimi A, Zarrini G, Dehghan G, Iranshahi M. Galbanic acid from Ferula szowitsiana enhanced the antibacterial activity of penicillin G and cephalexin against Staphylococcus aureus. Biol Pharm Bull, 2007; 30:1805-1807.
23. Yin W, Akala EO, Taylor RE. Design of naltrexone-loaded hydrolyzable crosslinked nanoparticles. Int J Pharm, 2002; 244:9-19.
24. Barar J, Javadzadeh AR, Omidi Y. Ocular novel drug delivery: Impacts of membranes and barriers. Expert Opi Drug Del, 2008; 5:567-581.
25. Sparreboom A, Scripture CD, Trieu V, Williams PJ, De T, Yang A, Beals B, Figg WD, Hawkins M, Desai N. Comparative preclinical and clinical pharmacokinetics of a Cremophor-free, nanoparticle albumin-bound paclitaxel (ABI-007) and paclitaxel formulated in cremophor (Taxol). Clinical Cancer Res, 2005; 11:4136-4143.
26. Wu Y, Loper A, Landis E, Hettrick L, Novak L, Lynn K, Chen C, Thompson K, Higgins R, Batra

U, Shelukar S, Kwei G, Storey D. The role of biopharmaceutics in the development of a clinical nanoparticle formulation of MK-0869: A Beagle dog model predicts improved bioavailability and diminished food effect on absorption in human. Int J Pharm, 2004; 285:135-146.
27. Alkhatib R, Hennebelle T, Joha S, Idziorek T, Preudhomme C, Quesnel B, Sahpaz S, Bailleul F. Activity of elaeochytrin A from Ferula elaeochytris on leukemia cell lines. Phytochemistry, 2008; 69:2979-2983.
28. Moghimi SM, Hunter AC, Murray JC. Long-circulating and target - specific nanoparticles: theory to practice. Pharmacol Rev 2001; 53:283-318.
29. Liu SQ, Tong YW, Yang YY. Incorporation and in vitro release of doxorubicin in thermally sensitive micelles made from poly(N-isopropylacrylamide-co-N,N-dimethylacrylamide)-b- poly(D,L-lactide-co-glycolide) with varying compositions. Biomaterials, 2005; 26:5064-5074.
30. Gupta AK, Madan S, Majumdar DK, Maitra A. Ketorolac entrapped in polymeric micelles: Preparation, characterisation and ocular anti-inflammatory studies. Int J of Pharm, 2000; 209:1-14.