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RESEARCH ARTICLE

T1107 polymer expressed potential anti-tumor effects against HepG2 cell line

El-Refaie Kenawy[1], Mohamed L. Salem[2,3], Noura E. Sanoh[1,3] and Mohamed M. Azaam[1]

[1]Chemistry Department, Faculty of Science, Tanta University, Tanta, Egypt
[2]Zoology Department, Faculty of Science, Tanta University, Tanta, Egypt.
[3]Center of Excellence in Cancer Research, Tanta University Educational Hospital, Tanta University, Tanta, Egypt

ABSTRACT

Background: Polymeric nanoparticles (NPs) are obtained naturally, semi-synthetically or synthetically by a polymerization reaction. Tetronics based ethylene oxide-propylene oxide copolymers have gained a great of interest. It demonstrated interactions with cell membranes with potential for developing new biomaterials for application in nanomedicine . **Aim:** The primary aim of the current study is to test the direct anticancer activity of unmodified tetronic (T1107), P-HB+aminated tetronic and N, N DMAB+aminated tetronic polymersand the associated by apoptosis and cell cycle. **Materials and Methods**: Synthetic polymers were prepared and characterized to confirm the modification. Anti-tumor activity was examined *in vitro* using human hepatocellular carcinoma (HepG2) cell line. Cell viability (MTT), cell cycle and apoptosis were evaluated by flow cytometry. **Results**: Unmodified tetronic (T1107) decreased HepG2 viability (by 30%) than untreated HepG2 cells. Treatment with P-HB+aminated tetronic and N,N DMAB+aminated did not give a significant effect on Hepg-2 cells. Treatment of HepG2 with unmodified tetronic (T1107) induced cell cycle arrest at G0 phase (39.4%), while P-HB+aminated tetronic and N,N DMAB+aminated tetronic induced cell cycle arrest at G1 phase (by 54.8% and 48.2%, respectively as compared to treatment with the doxorubicin (DOX) as a reference drug, which induced Hepg-2 cell cycle arrest at GO phase (by 39.1%). The unmodified tetronic (T1107) increased the numbers of late apoptotic cells (by 49.4%), while P-HB+aminated tetronic and N,N DMAB+aminated tetronic did not induced significant apoptosis. **Conclusion**:.Unmodified Tetronic T1107 induces an anticancer effect more than modified Tetronic polymers.

Keywords: Apoptosis, Cancer**,** Cell Cycle, Tetronic, Polymer

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Correspondence to

El-Refaie Kenawy, Ph.D
[1]Chemistry Departement, Faculty of Science Tanta University, Tanta, Egypt
Tel.01222372276
Email:ekenawy@yahoo.com

INTRODUCTION

In recent years, poloxamers and poloxamides have received increasing attention in the pharmaceutical field, mainly for their advantages as potential nanosystems (Ref). Thereby, in order to create new alternative treatments for current pathologies, like cancer, alterations in the poloxamine could promote the ability to incorporate a drug or a nucleic acid molecule, this nanosystem has the potential to be used in cancer treatment (Ref). Block copolymers contain at least two incompatible blocks, so they form solid-state microdomains and self-assemble in selective solvents (Bahadur 2001, Riess 2003), these characteristics, combined with advances in polymerization technology, make polymeric surfactants very useful in the material (Nakashima and Bahadur. 2006). Tetronics® is a commercially available poly ethylene oxide (PEO) poly propylene oxide (PPO) block copolymer with unique temperature-dependent micellization, surface activity, and reversible thermal rheological behavior(Gonzalez-Lopez et al .2008). Due to their low immunomodulatory activity and non-toxic effect, some of these copolymers have now been approved by the Food and Drug Administration (FDA) (Singh-Joy and McLain 2008). Their emerging applications in the manufacture of mesoporous materials (Sang

and Coppens. 2011, Chen and Chang. 2014) synthesis of nanoparticles (Habas et al. 2004, Singh et al. 2014) and nanocarriers as drug delivery systems (Hedberg et al. 2004, Oh et al. 2004, Csaba et al. 2005, Sezgin et al. 2006, Fernandez et al.2008) presented them as valuable biomaterials with potential medical applications (Hamley, 1998, Alexandridis and Lindman 2000). Tetronics® (also known as poloxamines) present an X-shaped structure made of an ethylenediamine central group bonded to four chains of PPO–PEO blocks. Tetronics® are synthesized by the sequential reaction of the acceptor ethylenediamine molecule first with propylene oxide (PO) and then with ethylene oxide (EO) precursors, resulting in a four-arm PEO-terminated molecular structure. The unique structure of Tetronics® provides them with multistimulus responsiveness.

Chemotherapy as Doxorubicin (DOX) is a chemotherapeutic drug that has been around since the late 1960s and is considered one of the most powerful broad-spectrum antitumor drugs. It's often used to treat a variety of cancers, including leukemia, lymphomas, soft-tissue sarcomas, and solid tissue sarcomas (Lefrak et al. 1973). There are more than 100 diverse chemotherapy drugs that cause diverse common side impacts such as bone marrow concealment (Khouri et al. 2008), leucopenia shows up at the 10th day of the chemotherapeutic course whereas thrombocytopenia after 10-14 days(Hadland B.K., Longmore G.D. 2009), and spewing foot, weakness, queasiness, handerythro-dysesthesia, cardiotoxicity. Moreover, thromboembolism, pericardial thickening, or cardiac arrhythmias (Chen and Di. 2016). The present study aimed to test the antitumor effect of Unmodified Tetronic (T1107), synthetic P-HB+Aminated Tetronic and, on HepG2 cells in-vitro and to assess their antitumor effects in-vitro determining whether these unmodified and modified polymers affect apoptosis and cell cycle of HepG2cells compared to doxorubicin.

MATERIALS AND METHOD

Chemicals and reagents

Tetronic 1107 (T1107) (Average molecular weight: 15000 g/mol) was purchesd from BASF Corporation (New Jersey, USA). Chloroacetyl chloride and Glacial acetic acid were obtained from El-Gomhouria Chemicals Company (Cairo, Egypt). Pyridine was obtained from El-Nasr Pharmaceutical Chemicals (Cairo, Egypt). p-Phenylene diamine was purchased from Acros Organics (Belgium). p-Hydroxybenzaldehyde, and p-dimethyl amino benzaldehyde were purchased from Aldrich (USA). All aldehydes were used without further purification. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] reagent were purchased from Millipore, Merck, Germany.Dulbecco's modified Eagle's medium (DMEM)and sterile phosphate buffered saline were purchased Lonza, USA, fetal bovine serum were purchased from Life Science Production, UK.

Cancer cell line

Human Hepatocellular Carcinoma (HepG2) cell line was purchased from Medical Technology Center, Medical Research Institute, Alexandria University, Egypt

Synthesis of Schiff base of T1107
Chloroacetylation of T1107

In a round flask (100 ml), 19 ml of Pyridine (240 mmol) was added to a solution of T1107 (10g, 20 mmol) in dry ethanol and the mixture was cooled to 0 oC. Chloroacetyl chloride (19 ml, 240 mmol) was added to the cold mixture dropwise with vigorous stirring (DAIHAN MaXtir™ 500S Hi-performance Digital Magnetic Stirrers, SRICO, South Korea). The reaction mixture was stirred at 0^{o}C for 3h and at room temperature for further 48h. The excess ethanol was removed by a rotary evaporator (IKA RV 10V Digital Rotary Evaporator, 115VAC, Germany). The precipitate was washed with diethyl ether and the product was dried under vacuum (Model VD 53, BINDER, Germany) for 10h (Figure.1).

Amination of chloroacetylated T1107

To a solution of p-phenylenediamine (13g, 120 mmol) in dry ethanol, chloroacetylated T1107 (5g, 6 mmol) were added portionwise. After the addition was completed, the system was fitted to reflux at 80^{o}C for 4 days with continuous stirring. The product was washed with ethanol to remove the unreacted diamine. The product

was then dried under vacuum for 48h (El-Safty et al. 2021).

Modification of the aminated tetronic with p-Dimethylamino benzaldehyde

A mixture of p-(dimethylaminobenzaldehyde) (1.06g, 7.13 mmol), aminated tetronic (1g, 0.89 mmol), and 1mL glacial acetic acid in 15mL methanol was stirred at room temperature for 48h. The system was fitted to reflux for 10h at 80°C. The product was filtered off and washed with methanol to remove the unreacted species. The product was collected as a dark red powder and dried under a vacuum oven at room temperature for 48h (El-Safty et al. 2021).

Modification of aminated tetronic with p-hydroxy benzaldehyde

A mixture of 4-hydroxybezaldehyde (0.87g, 7.13 mmol), aminated tetronic 1107 (1g, 0.89 mmol) and 1mL glacial acetic acid in 15 mL methanol was stirred at room temperature for 48h. The system was fitted to reflux for 10h at 80°C. The product was filtered off and washed with methanol to remove the unreacted species. The product was collected as dark orange powder and dried under vacuum at room temperature for 48h (El-Safty et al. 2021).

Measuring Cell Viability *in vitro* Using MTT

Adherent cells were harvested with 2 ml trypsin-EDTA 0.25% then counted by hemocytometer. After that cells were seeded into 96-well plates in 100 µl (5000 cell/well) for each well, the cells were then incubated in 5% CO2 at 37°C incubator for 24h. Stock solutions of Unmodified Tetronic (T1107), synthetic P-HB+Aminated Tetronic and N,N DMAB+ Aminated Tetronic (50,45,40,35,30,25,20,15, 10,5 mg/ml) were prepared based on optimal concentration of these polymers as used in drug delivery as a carrier(20mg/ml), by dissolving different concentration of the polymers in DMF (0.1%), while Doxorubicin concentration (ref. drug) was (1mg/ml). The plates were incubated for 24 hrs. at 37°C and in 5% CO2. After 24h of incubation, the medium was discarded from the plates and then the plates were washed with 100 µl PBS, then the cells were treated with 50µl of each polymers concentration diluted in 200µl of complete media, the plates were incubated for 24 h, MTT assay was performed for determining the cytotoxicity by adding 25 µl of MTT (5mg/ml) to each well and the plates were then incubated for 4. Then, cells were washed with PBS and 150µl DMSO was added to each well. The absorbance was measured at 545 nm using Plate Reader. The cell viability analysis was performed using Graph Pad Prism 6.0.

Measuring Apoptosis of HepG2 cells by Flow Cytometry

Based on the results of the cytotoxicity assay, HepG2 cells were collected from a 6-well plate previously treated with the stock solution of 5mg/ml (with final concentration 1.25mg/ml) of Unmodified Tetronic (T1107), synthetic P-HB Aminated Tetronic and N,N DMAB Aminated Tetronic. HepG2cells were washed twice with ice-cold PBS, the cell density was calculated, and the cells were re-suspended in 1× annexin-binding buffer to obtain a final density of 1×10^6 cells/ml. Then, 100µl of the cell suspension was placed into 1.5-ml Eppendorf tubes and5µl annexin V-fluorescein isothiocyanate (FITC) and 1µl PI (100µg/ml) working solution was added. Stained HepG2 cells were then incubated at room temperature for 15 min followed by the addition of 400 µl of 1× annexin-binding buffer with gentle mixing; then, the samples were kepton ice. The cells were then analyzed by flow cytometry.

Measuring Cell Cycle of HepG2 cells by Flow Cytometry

Based on the results of cytotoxicity assay, HepG2 cells were harvested from a 6-well plate previously treated with the stock solution of 5mg/ml (with final concentration 1.25 mg/ml), synthetic P-HB Aminated Tetronic and N,N DMAB Aminated Tetronic. HepG2 cells were prepared at a concentration of 1×10^6 cells/ml, washed twice with ice-cold PBS, and fixed with 70% ethanol at 4°C overnight. The fixed cells were re-suspended in 300– 500 µl PI/Triton x100 staining solution (1000µl of 0.1% Triton+ 40µl PI+ 20µl RNAse), for30 min at 37°C in the dark. The cells were then centrifuged at 1000×*g*, and the number of cells at the different phases of the cell cycle was analyzed using flow cytometry (BD FACSCanto IIflow cytometry, BD Biosciences, USA) and the data were analyzed using FlowJo software

RESULTS

Measuring modification of the aminated T1107 with p-hydroxy benzaldehyde by FT-IR spectra

The FT-IR spectrum of T1107 Schiff base (IV) showed the disappearance of the primary amino group, instead a strong peak appeared at 1603 cm^{-1} which belongs to the azomethine group (C=N) due to the reaction of aminated T1107 with p-hydroxy benzaldehyde that confirms the formation of the Schiff base (Figure.2).

Modification of the aminated T1107 with dimethyl amino benzaldehyde

The FT-IR spectrum of T1107 Schiff base (V) showed the disappearance of the primary amino group, instead a strong peak appeared at 1599 cm^{-1} which belongs to the azomethine group (C=N) due to the reaction of aminated T1107 with p-hydroxy benzaldehyde that confirms the formation of the Schiff base (Figure.2).

Sample size characterization by **zetasizer analysis**

Size characterization of samples was made by dynamic light-scattering (DLS) measurements using the Zetasizer Malvern, the result show distribution of and N,N DMAB+Aminated Tetronic, of P-HB+Aminated Tetronic in particles size 1339nm, 2121 nm, respectively in a volume equal to 100 ml of distl water, while the unmodified Tetronic particle size is 5.6 nm (Figure.3).

Effect of Unmodified Tetronic (T1107) and modified Aminted Tetronic polymers on HepG2 cells viability using MTT

Treatment of HepG2 cells with Unmodified Tetronic (T1107), P-HB+Aminated Tetronic and N,N DMAB+Aminated Tetronic compared to DOX treatment however there was some aggregations in media (Figure 4), the different concentrations of P-HB+Aminated Tetronic and N,N DMAB+Aminated Tetronic did not show any effect on the cells viability, while the low concentration (stock conc=5mg/ml which diluted into 1.25mg/ml) of Unmodified Tetronic (T1107) showed that reduced HepG2 cells viabilty to (67%) compared to negative control Hepg-2 cells (100%) and Dox (45%) (Figure 5).

Effect of unmodified and modified Tetronic (T1107) polymer on apoptosis of HepG2 cells *in vitro*

Healthy and apoptotic cell percentage of HepG2 cells was analysed using flowcytometry after treatment with Unmodified Tetronic (T1107), P-HB+Aminated Tetronic and N,N DMAB+Aminated Tetronic and phenotyping distribution of healthy and late apoptotic cells (Figure 6) and % of healthy,early apoptotic, late apoptotic cells and necrotic cells (Figure 7). The results showed that treatment with Unmodified Tetronic (T1107) increased the late apoptotic cells % to 49.2% compared to N,N DMAB+Aminated Tetronic and P-HB+Aminated Tetronic and DOX, 1.77%, 0.65 % and 29.2% respectivly.

Effect of unmodified and modified Tetronic (T1107) polymer on cell cycle of HepG2 cells *in vitro*

Nuclear DNA content of Hepg-2 cells was analyzed using flow cytometry after treatment with P-HB+Aminated Tetronic and N,N DMAB+Aminated Tetronic and unmodified Tetronic at (5 mg/ml) respectively, compared to free DOX as reference drug. results showed that DNA content of Hepg-2 cells undergoes different phases (sub G0, G1, S, and G2M) of the cell cycle before mitotic division and graphs of fractional DNA content (PI fluorescence, X-axis) versus cell counts (Y-axis) are displayed in Figure 8. Cell cycle distribution of HepG2cells from Hepg-2 cells treated with Unmodified Tetronic (T1107)induced cell cycle arrest at G0 phase (39.4%)while, P-HB+Aminated Tetronic and N,N DMAB+Aminated Tetronic arrested the cell cycle at G1 phase 54.8% and 48.2% respectively compared to free DOX, which induced HepG2 cell cycle arrest at GO phase 39.1% (Figure 8).

Statistical analysis

Numerical data obtained from each experiment were expressed as mean ± SE and statistical differences between experimental and control groups were assessed using One-way Analysis of Variance (ANOVA). Graph Pad Prism (Graph Pad Software, Inc., San Diego, CA) was used to analyze P values. P values <0.05 were considered statistically significant.

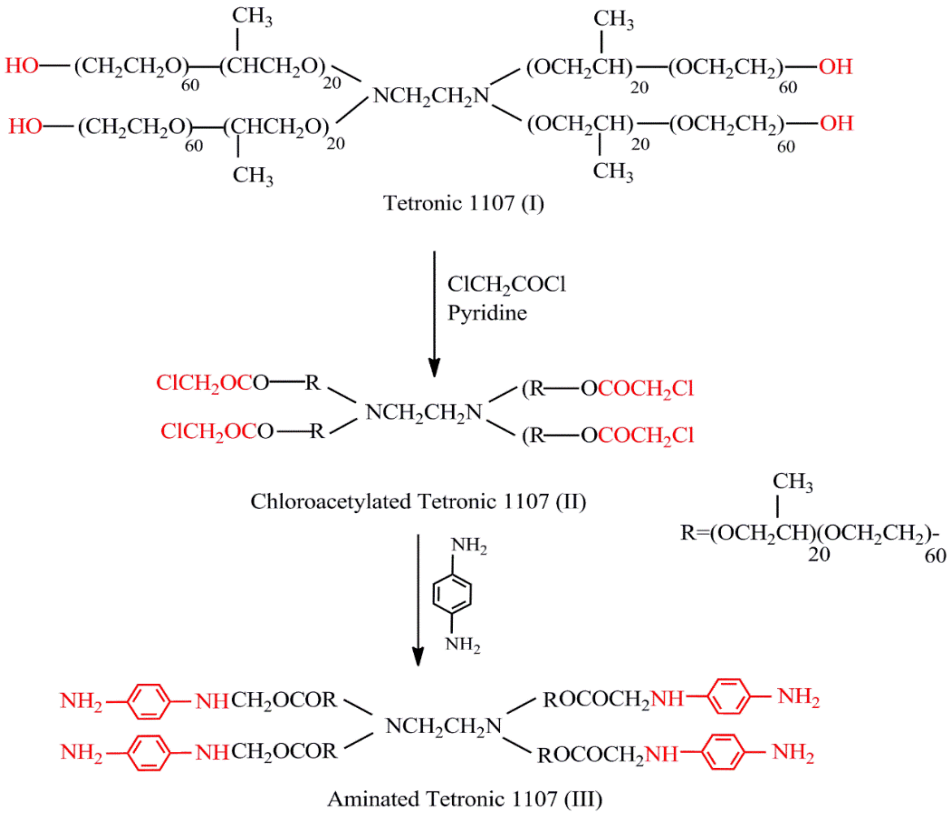

Figure1. Scheme of Synthesis of Aminated T1107

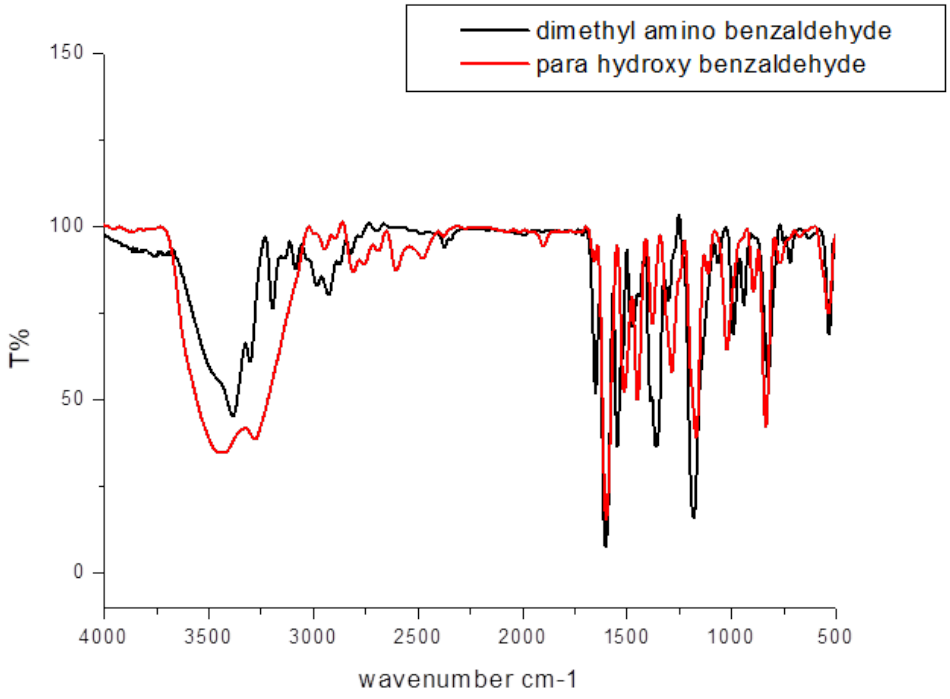

Figure 2. FT-IR spectrum of Schiff base aminated tetronic+ dimethyl amino benzaldehyde (DMAB+Aminated Tetronic) and aminated tetronic+ para hydroxyl benzaldehyde (PHB+Aminated Tetronic).

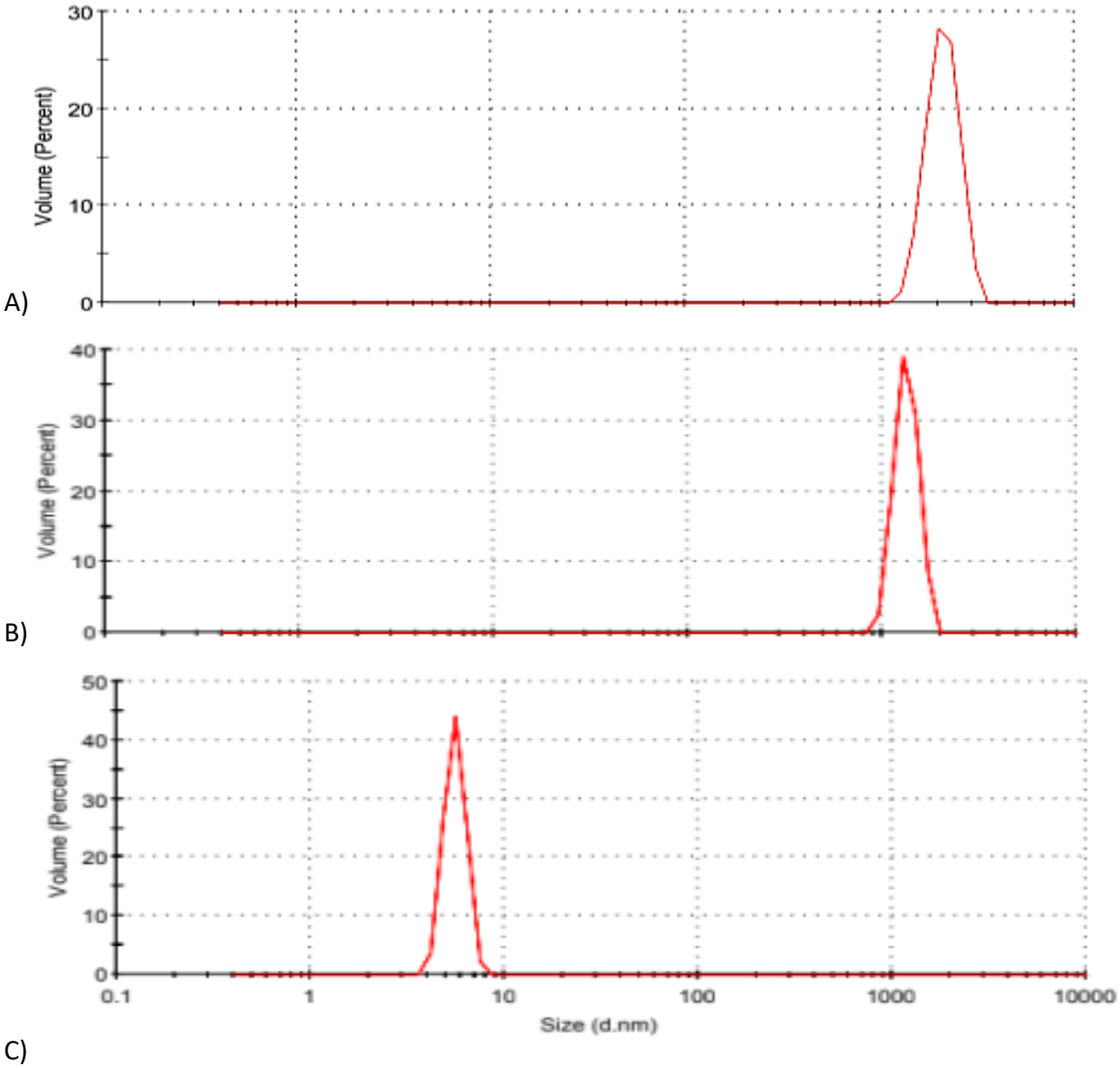

Figure 3. Particle size distribution of P-HB+Aminated Tetronic, N,N DMAB+Aminated Tetronic and Unmodified Tetronic measured on a Zetasizer Marvlen, (A) P-HB+Aminated Tetronic, (B) N,N DMAB+Aminated Tetronic, (C) Unmodified Tetronic(T1107).

DISCUSSION

Since, DOX is widely used as a chemotherapeutic drug for treating a variety of cancers such asliver cancer. However, it associates with significant side effects. The present study wasundertaken to evaluate the capability of Unmodified Tetronic (T1107), N,N DMAB+Aminated Tetronic and P-HB+Aminated Tetronic nanopolymer as anticancer materials, if any, and to compare the anti-tumor effects of these materials to DOX, using Human Hepatocyte Carcinoma (HepG2) cell line in vitro.

According to (El-Safty et al. 2021) N,N DMAB+Aminated Tetronic and P-HB+Aminated Tetronic were synthesized by Schiff Base of Tetronic 1107. The FT-IR spectrum of T1107 Schiff base showed the disappearance of the primary amino group azomethine group (C=N) due to the reaction of aminated T1107 with p-hydroxy benzaldehyde forming P-HB+Aminated Tetronic, and reactionwith p-Dimethylamino benzaldehyde forming N,N DMAB+Aminated Tetronic, this chemical modification could led to greater stability of the modified copolymer compared to the unmodified Tetronic (Jonathan et al., 1998). The free amino groups caused an increase in the phagocytosis in experiments carried out with HepG2 cells.

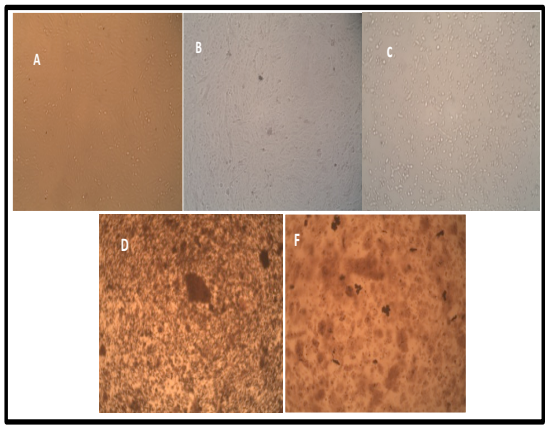

Figure 4. Effect of modified Tetronics on Hepg-2 cells (A) Hepg-2 cancer cells, (B) Doxorubic, (C) Unmodified Tetronic, (D) N,N DMAB+Aminated Tetronic, (E) P-HB+Aminated Tetronic in on cancer cells viability. HepG2 cells were cultured in vitro for 24 hr and then assessed under inverted microscope.

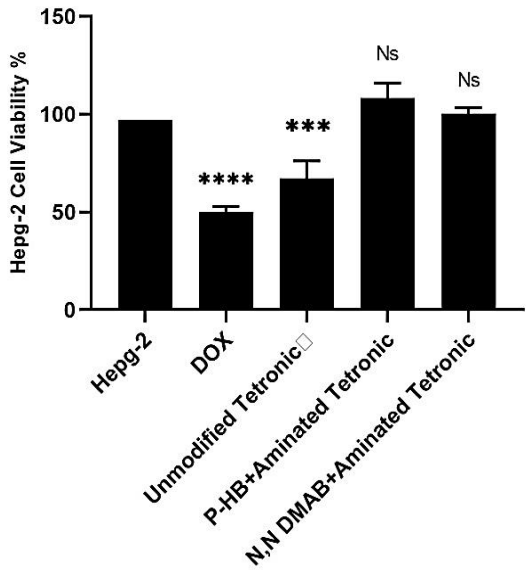

Figure 5. The effect of modified Tetronic on HepG2 viabillty. HepG2cells were seeded in complete DMEM medium at (37 C, 5%CO2) and treated with N,N DMAB+Aminated Tetronic and (F) P-HB+Aminated Tetronic and HepG2cell viability was measured using MTT assay. Data were represented as mean ± SE (n=3). ***p=0.0006, **** P < 0.0001, Ns (Not significant) statistically significant comparison of control group and other treated group.

When the amine groups were capped (p-hydroxy benzaldehyde and p-Dimethylamino benzaldehyde, the coating was 1-2 µm, the surface recovered the anionic character, and the uptake by the cells was reduced, although it was still higher than for Unmodified Tetronic nanospheres. Minor differences were observed regarding biodistribution: tetramine poloxamine enhances splenic uptake, while the capped poloxamine derivative reverts this effect and slightly enhances the fraction of nanoparticles at blood 3 hours after injection (Jonathan et al .1998).

The zetasizer showed the presence of the unmodified Tetronic (T1107) in nano size and itdid not exceed 10 nm, It is well known that particle size is crucial for biomedical applications during circulation and biodistribution inside the living system. For nanomedicine, particles smaller than 10 nm can be easily removed from the kidney or by extravasation, while larger particles have an adverse effect on diagnostic sensitivity and therapeutic effects, and are easier to remove by the reticuloendothelial system. Furthermore, compared to unmodified Tetronic (T1107), the particle size range of DMAB+ Aminated Tetronic and PHB+ Aminated Tetronic is 12 µm, andthe effect of these modified polymers as antitumor materials is not significant. Cytotoxicity analysis showed that, compared to DMAB+ Aminated Tetronic and PHB + Aminated Tetronic, unmodified tetronic T1107 showed higher cytotoxicity to HepG2 cancer cells at a stock concentration

of 5mg/ml (with final concentration 1.25 mg/ml). This effect is due to the existence of free functional groups with unmodified tetronic, which may be due to the induction of cytotoxicity due to the reaction with specific cell receptors. In DMAB+ Aminated Tetronic and PHB+ aminated Tetronic, functional groups limit main stability Prevent reaction with cell receptors. Several anticancer compounds have been shown to have growth inhibitory effects by blocking the cell cycle at specific checkpoints of the cell cycle or by inducing apoptosis or the collective effect of cycle arrest and apoptosis (Gerard EI, Karen VH. 2001). Therefore, this study analyzed the effects of unmodified Tetronic T1107, DMAB + aminated Tetronic, and PHB + aminated Tetronic on these parameters. They exhibit different characteristics in the cancer cell cycle.

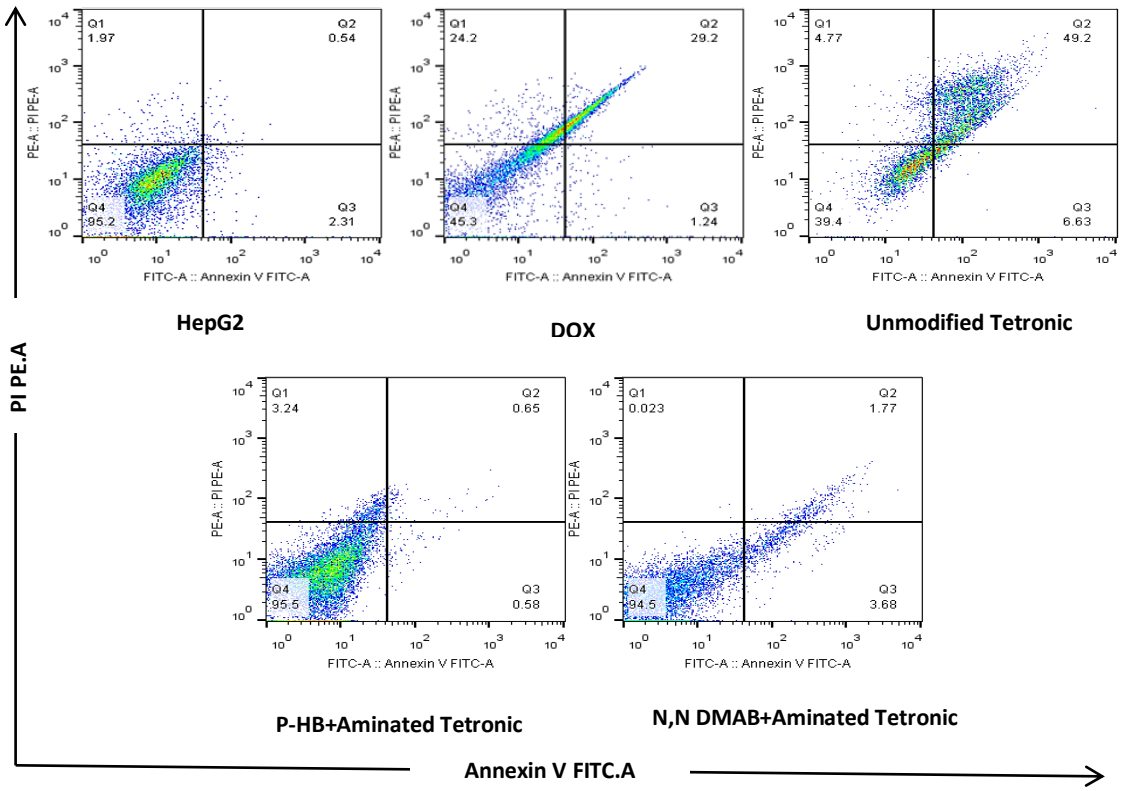

Figure 6. The effect of modified Tetronic on HepG2 cell apoptosis. HepG2cells were treated with modified pluronics for 48h then phenotyping distribution of HepG2 cells and % of healthy, early apoptotic, late apoptotic cells and necrotic cells were assessed.

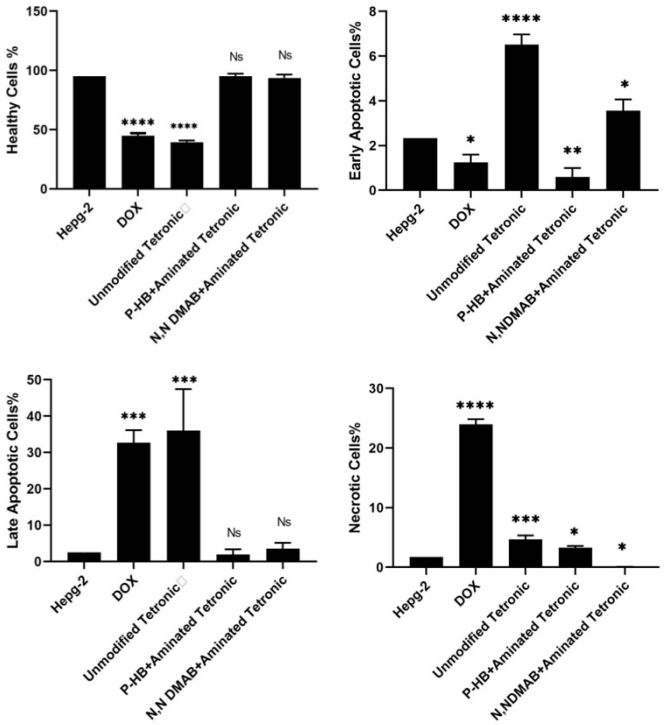

Figure 7. The effect of modified Tetronic on HepG2 cell apoptosis. HepG2 cells were treated with modified Tetronic for 48h then phenotyping distribution of Hepg-2 cells and % of healthy, early apoptotic, late apoptotic cells and necrotic cells were assessed. Data were represented as mean ± SE (n = 3). * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $P \leq 0.0001$ statistically significant comparison of control group and other treated.

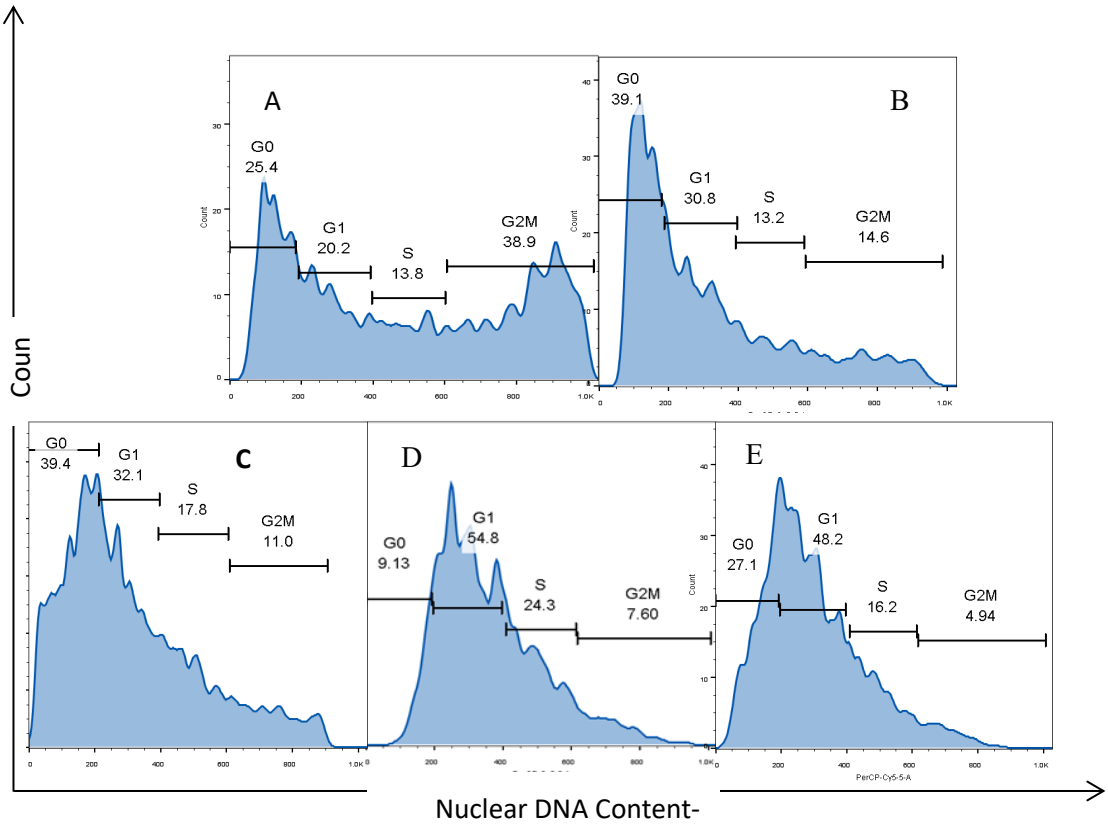

Figure 8. Nuclear DNA contents of Hepg-2 cells after treatment with modified and unmodified Tetronic . HepG2 cells were treated with modified polymers for 48 hr and then HepG2cell cycle was analyzed. (A) Hepg-2 cancer cells, (B) Doxorubic, (C) Unmodified Tetronic, (D) N,N DMAB+Aminated Tetronic, (E) P-HB+Aminated Tetronic.

Unmodified Tetronic T1107 stops about 39.4% of cancer cell cycle inG0 phase.t This means that the cells remain metabolically active in this phase but do not proliferate; the cells are not actively dividing or preparing to divide. On the other hand, when the cells enter the cell cycle they show the different phases of cells cycle as shown after treatment with combination of N,NDMAB and aminated Tetronic or after combination of PHB and Aminated Tetronic.

With regard to apoptosis of HepG2 cells, the current study revealed that treatment with the unmodified Tetronic T1107 showed higher numbers of Hepg-2 in late apoptosis. By contrast, treatment with DMA/Aminated Tetronic and P-HB+Aminated Tetronic did not show a significant effecton Hepg-2 cells as compared with free DOX. These data suggest that the anti-cancer effects ofthe unmodified Tetronic T1107 are mediated by both arresting the cell cycle of HepG-2 as well as inducing apoptosis.

CONCLUSION

Unmodified Tetronic T1107 induces an anticancer effect more than modified Tetronic polymers. Further studies are needed to optimize the beneficial effects of the obtained data and to understand the underlying mechanisms.

ABBREVIATIONS

- DOX: Doxorubicin
- N,N DMAB+Aminated Tetronic: N,N Dimethyl Amino Benzaldehyde Aminated Tetronic
- P-HB+Aminated Tetronic: Para Hydroxy Benzaldehyde Aminated Tetronic

AUTHORS' CONTRIBUTION

All authors participated in the design of the study and performed the statical analysis, all authors contributed to the manuscript revision, all autours approved the final manuscript.

CONFLICT OF INTEREST

All authors declare no conflicts of interest.

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REFERENCES

Alexandridis P, Lindman B, 2000. Amphiphilic Block Copolymers: Self-Assembly and Applications; Elsevier:Amsterdam, The Netherlands.

Bahadur P. 2001, Block copolymers-their microdomain formation (in solid state) and surfactant behaviour (in solution). Curr. Sci. 80, 1002–1007.

Chen Z, DiA (2016). Cardiotoxicity associated with targeted cancer therapies. Moecular and clinical oncology 678-41:675.

Chen JK, Chang CJ. 2014, Fabrications and applications of stimulus-responsive polymer films and patterns on surfaces: A review. Materials. 7, 805–875.

Csaba N, Caamaño P, Sánchez A, Domínguez F, Alonso MJ Plga. 2005, Poloxamer and plga: Poloxamine blend nanoparticles: New carriers for gene delivery. Biomacromolecules. 6, 271–278. [CrossRef] [PubMed].

El-Safty Samy, Kenway El-Refaie, Azaam Mohamed, Mahmoud Yehia, Khattab Samar, 2021. Mechanical properties and fluoride release of glass ionomer cement with enhanced antimicrobial activity derived from addition of schiff base of tetronic 1107- egyptian dental journal.

Fernandez-Tarrio, M, Yañez, F, Immesoete K, Alvarez-Lorenzo C, Concheiro A. 2008, Pluronic and tetronic copolymers with polyglycolyzed oils as self-emulsifying drug delivery systems. AAPS PharmSciTech, 9, 471–479.

Gerard EI, Karen VH. 2001. Proliferation, cell cycle and apoptosis in cancer. Nature 411:342–348.

Gonzalez-Lopez. J, Alvarez-Lorenzo. C, Taboada P et al (2008). Selfassociative behavior and drug-solubilizing ability of poloxamine(tetronic) block copolymers. Langmuir 24:10688–10697.

Habas JP, Pavie E, Perreur C, Lapp A, Peyrelasse J. 2004, Nanostructure in block copolymer solutions:Rheology and small-angle neutron scattering. Phys. Rev. E,70, 061802.

Hadland BK, Longmore GD. 2009. Erythroid-stimulating agents in cancer therapy: potential dangers and biologic mechanisms. J Clin Oncol 27:4217-4226.

Hamley IW. 1998, The Physics of Block Copolymers; Oxford University Press: New York, NY, USA, Volume 19.

Hedberg EL, Shih CK, Solchaga LA, Caplan AI, Mikos AG. 2004, Controlled release of hyaluronan oligomers from biodegradable polymeric microparticle carriers. J. Control. 100, 257–266.

Jonathan C. Neal, Snow Stolnik, Etienne Schacht, El Rafaie Kenawy, Martin C. Garnett, Stanley S. Davis, Lisbeth Illum: 1998. In vitro displacement by rat serum of adsorbed radiolabeled poloxamer and poloxamine copolymers from model and biodegradable nanospheres. J Pharm Sci 87, 1242-1248.

Jonathan C. Neal, Snow Stolnik, Martin C. Garnett, Stanley S. Davis, Lisbeth Illum: 1998. Modification of the copolymers poloxamer 407 and poloxamine 908 can affect the physical and biological properties of surface modified nanospheres. Pharm Res 15, 318-324 PMid:9523321.

Khouri S, Kotliroff A, Lishner M, Amital H. 2008. Imatinib-induced agranulocytosis in a patient with chronic myelogenous leukemia in remission. Isr bMed Assoc J 10:320-321.

Lefrak EA, Pita J, Rosenheim S. 1973. J.A.G. clinicopathological analysis of adriamycin cardiotoxicity. A Cancer 32: (11) 302–314.

Nakashima K, Bahadur P. 2006, Aggregation of water-soluble block copolymers in aqueous solutions: Recenttrends. Adv. Colloid Interface Sci. 123, 75–96.

Oh KT, Bronich TK, Kabanov AV. 2004. Micellar formulations for drug delivery based on mixtures of hydrophobic and hydrophilic pluronic® block copolymers. 2004, J. Control. Release, 94, 411–422.

Riess G. 2003. Micellization of block copolymers. Prog. Polym. Sci. 28, 1107–1170.

Sang LC, Coppens MO. 2011. Effects of surface curvature and surface chemistry on the structure and activity of proteins adsorbed in nanopores. Phys. Chem. Chem. Phys. 13, 6689-6698.

Sezgin Z, Yüksel N, Baykara T. 2006, Preparation and characterization of polymeric micelles for solubilizationof poorly soluble anticancer drugs. Eur. J. Pharm. Biopharm. 64, 261–268.

Singh,V, Khullar P, Dave PN, Kaura A, Bakshi MS, Kaur G. 2014. PH and thermo-responsive tetronic micelles for the synthesis of gold nanoparticles: Effect of physiochemical aspects of tetronics. Phys. Chem. Chem. Phys. 16, 4728–4739.

Singh-Joy SD, McLain VC (2008) Safety assessment of poloxamers 101, 105, 108, 122, 123, 124, 181, 182, 183, 184,185, 188, 212, 215, 217, 231, 234, 235, 237, 238, 282, 284, 288,31, 333, 334, 335, 338, 401, 402, 403, and 407, poloxamer 105evolution of drug delivery concept from inert nanocarriers to biological response modifiers. J Control Release 130:98–106.