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Summary of PhD Thesis

DEVELOPMENT OF TITANATE NANOTUBES SPECIFIED FOR PHARMACEUTICAL APPLICATIONS BASED ON QUALITY BY DESIGN AND ARTIFICIAL NEURAL NETWORK MODELLING

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QUALITY BY DESIGN GUIDED SYNTHESIS AND FUNCTIONALIZATION OF TITANATE NANOTUBES AS DRUG DELIVERY SYSTEMS

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ABBREVIATIONS

AA	Acrylic acid
ADMET	Absorption, distribution, metabolism, excretion, toxicity
AI	Artificial intelligence
ANN	Artificial neural network
APIs	Active pharmaceutical ingredients
BBB	Blood brain barrier
CA	Citric acid
CFE	Cold field emission
CMAs	Critical materials attributes
CNTs	Carbon nanotubes
CPPs	Critical process parameters
CQAs	Critical quality attributes
DLS	Dynamic light scattering
DMSO	Dimethylsulfoxide
DS	Design space
EMEM	Eagle's Minimum Essential Medium
FCS	Fetal calf serum
FT-IR	Fourier transform infrared
IVIVC	In vitro-in vivo correlation
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NaoH	Sodium hydroxide
Na_2SO_3	Sodium sulfite
$Na_2S_2O_8$	Sodium persulfate
NPs	Nano particles
PAA	Poly acrylic acid
PBS	Phosphate buffered saline
PEG	Polyethylene glycol
PEI	Polyethyleneimine

PK	Pharmacokinetics
QbD	Quality by design
QTPPs	Quality target product profiles
REM	Risk estimation matrix
RES	Reticuloendothelial system
RMSE	Root mean square error
ROS	Reactive oxygen species
SAR	Structure activity relationship
SEM	Scanning electron microscopy
SSA	Specific surface area
TCA	Trichloroacetic acid
TEM	Transmission electron microscopy
TiO_2	Titanium dioxide
TNTs	Titanate nanotubes
VLS	Vapor-Liquid-Solid

1. INTRODUCTION

Nowadays, an accelerated transformation is taking place to replace the use of traditional bulk materials with smart, predesigned nanomaterials in all aspects of modern life. The impressive performance of these newly introduced nanomaterials is shaping the future of science and revolutionizing the traditional concepts of industry and research. For this reason, all scientific disciplines should keep up with this tremendous evolution and each field must explore the opportunities to invest in these newly invented tools for its own benefit.

Titanate nanotubes (TNTs) are one of these newly invented materials that were introduced as a superior replacement of their organic counterparts (carbon nanotubes) and proved to be efficient tools in various fields and purposes. Several research works demonstrated their effectiveness in medical, industrial, and chemical disciplines such as in dental implants and orthopaedics, chemical catalysis, biofuel synthesis, solar cells and water purification. This outstanding performance in these different areas raise the question about their potential role in the field of pharmaceutics.

Hence, TNTs are attracting significant interest in the pharmaceutical community as they could be a novel platform for pharmaceutical applications especially in the field of drug delivery. Their attractiveness arises from the remarkable properties they could offer such as advantageous tubular geometry, large specific surface area (SSA) compared to their spherical counterparts, hydrophilic nature, nano-size undetectable for the reticuloendothelial system, good biocompatibility and mechanical strength. This unique package of properties is of a fundamental importance and considered as a key feature for their prospective use as drug carriers. However, some of their characteristics may hinder their successful application in healthcare, and this could be overcome by functionalizing their surface which can enhance their excellent properties and modify them to meet pharmaceutical requirements.

2. AIM AND OBJECTIVES

The first part of the work aimed to create a roadmap for the successful development of TNTs specified for pharmaceutical applications, with the combination of QbD tools and artificial intelligence (ANN modelling).

This part tested the following hypothesis:

Sufficient data could be derived from literature to create a neural model for prediction of full physicochemical and toxicological profile of TNTs based on the corresponding synthesis parameters. The obtained model can be used to select the proper parameters to synthetize TNTs suitable for pharmaceutical applications.

The second part of the study aimed to further modify the characteristics of the obtained TNTs by functionalization, and to test the hypothesis that functionalization can be more effective via creating an extended and flexible arm on their surface using carboxylic acids. Enriching TNTs surface with reactive carboxylic groups could facilitate subsequent grafting of numerous molecules/drugs or furthermore attaching biologically active substances that could not be directly linked to the original surface of TNTs.

This approach could be an appropriate way to create functional nanomaterials facilitating their further functionalization and would result in tailoring TNTs surface chemistry to enhance their characters or adapt them to fit well with the pharmaceutical requirements.

In the following step polyethylene glycol (PEG) was selected as the functionalizing agent that will be bonded to the carboxylic arm due to its well-known biological benefits such as resisting non-

specific protein adsorption and thus the formation of protein corona, prolonging circulation time and reducing cytotoxicity.

3. MATERIALS AND METHODS

3.1. Materials

Three types of carboxylic acids: trichloroacetic acid (TCA), citric acid (CA) monohydrate (both from Molar, Chemicals Ltd., Budapest Hungary) and acrylic acid (AA) (Sigma-Aldrich Ltd., Budapest, Hungary) in addition to two types of Polyethylene glycol (PEG 600 and PEG 6000) (Fluka AG, Buchs, Switzerland) were used to functionalise the surface of TNTs. Sodium persulfate, sodium sulfite and sodium hypophosphite monohydrate reagents were also purchased from Sigma-Aldrich Ltd., (Budapest, Hungary).

3.2. Methods

3.2.1. Methods for collection literature data

QTPP, CQAs, CMAs, and CPPs were selected using previous experience and literature data. Relevant scientific databases: Web of Science, Scopus, PubMed, etc., were searched using the keywords titanate nanotubes, synthesis, hydrothermal treatment, drug delivery, toxicity, etc. Then, a database including the investigated CMAs, CPPs, and CQAs was created after critical evaluation and curation of the gathered data. This database was used for supporting the risk assessment process and served as basis to create the ANN model.

3.2.2. Risk assessment tools

Numerous quality tools were applied during the risk assessment. An Ishikawa diagram was used for the identification of CPPs and CMAs affecting different steps and profiles (TNT preparation, API incorporation, functionalization, and safety profile), REM containing interdependence rating of the identified parameters was created using a three-level scale (high (H), medium (M), or low (L) dependence), and they appeared in the interdependence tables using the colours: red, yellow, and green, respectively. The evaluation was made with LeanQbD software (Version 1.3.6., 2014 QbD Works LLC, Fremont, CA, USA). In the software, the qualitative three-level scale used for the estimation is linked to a selectable numeric scale which, at the end of estimation, gives the severity score of the evaluated risk factors based on mathematical calculations. After the categorization of the interdependence, the risk occurrence rating of CMAs/CPPs was made, applying the same three-grade scale (H/M/L) for the analysis. As the output of the initial RA evaluation, Pareto diagrams were generated by the software, presenting the numeric data and the rankings of CQAs and CPPs/CMAs according to their potential impact on the aimed final product.

3.2.3. ANN modelling

ANN modelling was performed using TIBCO Statistica v.14.0.1.25 (Tibco Software Inc., Palo Alto, CA, USA). The full dataset collected from the literature and two outputs were identified after careful data curation, in which the existing data enabled suitable modelling. The morphology and SSA of the obtained products were then handled as two different datasets for classification and regression-type modelling. The range of input variables was the same for both models. After setting the datasets, the minimum and maximum values of each input and output parameters were identified manually, and these cases were included into the training subset to avoid the need for extrapolation during testing or validation of the model. The remaining data were then randomly distributed into the training,

testing, and validation subsets, where the sizes of the training, testing, and validation subsets were 70%, 15%, and 15% of the full dataset, respectively.

Feed-forward, back-propagation multilayer perceptron networks were developed in both cases. The networks were trained with the BFGS algorithm. The possible range of the number of hidden neurons was set according to the following equation (Eq. 1):

$$1 \leq n \leq I + O + 1 \quad (1)$$

where *I* is the number of inputs, *O* is the number of outputs, and *n* is the number of hidden neurons; thus, the hidden neuron number varied from 1–20 for the classification and 1–12 for the regression network, respectively.

A multi start method including 10,000 networks was applied using the automated neural networks module of Statistica, including a training approach to screen the best performing network with different initialization patterns and activation functions for hidden and output neurons. The training was stopped when the root mean square error (RMSE) of the test dataset reached its minimum. The 10 best performing networks from each multi start run were retained for further analysis.

The prediction performance of the networks was evaluated based on network perfection, which is the mean R^2 of the observed vs. predicted data of each output neuron, and on the RMSE of predictions on the validation subset.

3.2.4. Preparation of TNTs

TNTs were prepared at the Department of Applied and Environmental Chemistry, University of Szeged, according to Sipos et al, using hydrothermal treatment method. Briefly, 120 g of NaOH were added to 300 mL of distilled water during continuous mixing, then 75 g of TiO_2 were added and mixed for 15 mins. This mixture was put in an autoclave at 185°C for 24 hrs then cooled at room temperature for 2 hrs, followed by cooling with cold water. The TNTs were finally washed with distilled water under vacuum using filter No:4.

3.2.5. Preparation of carboxylic acid functionalized TNTs

Functionalization of TNTs with TCA was performed by adding 3 g of TNTs to 90 ml of water in an ultrasonic bath for 1 hour until a homogenous suspension was obtained. This suspension was heated at 80°C in a condenser connected to nitrogen gas for 30 mins then TCA was added to the previous system and mixed for one day.

CA functionalized TNTs (CA-TNTs) were prepared by adding 0.5 g of TNTs to 15 ml of water containing 1.5 g of citric acid on a magnetic stirrer. This mixture was stirred for 30 mins to obtain a homogenous suspension then heated at 50°C with continuous stirring for 24 hrs.

AA functionalized TNTs (AA-TNTs) were prepared by mixing 1 g of TNTs with 28.8 g of acrylic acid, 32 g of hexane and 8 g of water then placed for sonication in an ultrasonic bath for 20 mins at room temperature. This mixture was stirred at room temperature on a magnetic stirrer for 2 days then separated by centrifugation at 12,000 rpm for 60 min at 8°C.

3.2.6. Functionalisation with PEG

CA-TNTs were further functionalized with PEG 600 by mixing CA-TNTs and PEG 600 at 130°C in a silicon oil bath for 24 hrs in an inert atmosphere condition by bubbling nitrogen gas then the mixture was cooled to room temperature and filtered.

AA/PEG 6000 functionalized TNTs were prepared by applying a two-step method, where first an AA-PEG copolymer was prepared according to Abo-shosha et al. and Ibrahim et al. followed by bonding the resulted copolymer with TNTs. Briefly, the AA-PEG 6000 copolymer was prepared by a polymerization reaction in a thermostatic water bath at 40°C under atmospheric oxygen. The polymerization medium of poly acrylic acid (PAA)/PEG was prepared by neutralizing 20% of AA (115.5 g) with the equivalent amount of an aqueous solution of 500 g/L NaOH followed by dissolving 40 g of PEG 6000. After that, 3.3 ml of 40 g/L sodium sulfite (Na_2SO_3) solution and 12.2 ml of 330 g/L sodium persulfate ($Na_2S_2O_8$) solution were added with stirring. An exothermic polymerization reaction commenced after an induction period with the evolution of water vapour followed by solidification of the polymerization medium. The latter was then cooled, disintegrated, oven dried at 105°C for 2 hrs, cooled, and kept over silica gel for at least 40 hrs before analysis. In the second step, this copolymer (50 g/L) was bonded with TNTs (5 g/L) after adding Na-hypophosphite (15 g/L) as a catalyst in this reaction.

All the prepared composites were subjected to a washing step to remove any residues adsorbed on the surface of TNTs and finally were dried in a drying oven (Memmert, Büchenbach, Germany).

3.2.7. Structural investigation

The determination of the success of functionalization procedure and the nature of TNT-carboxylic acid interactions were evaluated using an Avatar 330 FT-IR spectrometer (Thermo Fisher Scientific Ltd., Waltham, MA, USA). FT-IR measurements were conducted with a transmission E.S.P. accessory by using 128 scans at a resolution of 4 cm^{-1} and applying H_2O and CO_2 corrections. Spectragryph 1.2.16.1 software (Friedrich Menges, Obersdorf, Germany) was used to evaluate the results.

A DXR Dispersive Raman spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) equipped with a CCD camera and a diode laser operating at a wavelength of 780 nm was applied to perform Raman measurements, which were carried out with a laser power of 12 and 24 mW at 25 μm slit aperture size. The data were collected in the spectral range of 3407–24 cm^{-1} using photobleaching to compensate fluorescence of titanate. OMNIC 8 software was used for data collection, averaging the total of 20 scans and making the spectral corrections. For the removal of cosmic rays, a convolution filter was applied on the original spectrum using Gaussian kernel.

3.2.8. Morphology, size and surface charge investigations

The morphology and size of bare and functionalized TNTs were investigated by SEM (Apreo C, Thermo Fisher Scientific Ltd., Waltham, MA, USA) and TEM (FEI Technai G2 20 X-TWIN, Hillsboro, OR, USA). SEM was instrumented with a cold field emission (CFE) cathode. The system was used under 10^{-7} Pa ultra-high vacuum and the samples were maintained at room temperature and under 10^{-4} Pa vacuum during the characterization in the sample chamber. TEM images were taken at 200 kV of electron energy.

The hydrodynamic diameter and zeta potential measurements were done using dynamic light scattering with a Nano ZS zetasizer system (Malvern Panalytical Ltd., Malvern, Worcestershire, UK),

equipped with a 633 nm wavelength laser. The bare and functionalized TNT samples were prepared by dispersing them in different media (water, phosphate buffered saline (PBS) and a PBS based cell culture medium) with 30 min of ultrasonication and then 1 ml of each dispersion was placed in folded capillary zeta cells.

3.2.9. Cell viability studies

The direct toxicity of the newly functionalized TNTs was determined on two intact, non-cancerous cell lines (human embryo fibroblast (MRC5) and mouse embryonic fibroblast (NIH/3T3) cell lines) as well as on malignant human ovarian carcinoma (A2780) and two types of oropharyngeal squamous carcinoma cell lines (UPCI-SCC-131 and UPCI-SCC-154) by standard MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method. The MRC5, NIH/3T3, and A 2780 cell lines were purchased from the European Collection of Cell Cultures (ECACC, Salisbury, UK), while the two oropharyngeal cell lines from the German Collection of Microorganisms and Cell Cultures GmbH (Braunschweig, Germany). Each cell line was maintained at 37 ºC in a humidified atmosphere (containing 5% CO_2) in Eagle's Minimum Essential Medium (EMEM) supplemented with the appropriate amount of heat-inactivated fetal calf serum (FCS), non-essential amino acids, and 1% antibiotic-antimycotic mixture (penicillin-streptomycin), according to the manufacturer's recommendations. All media and supplements were obtained from Lonza Group Ltd. (Basel, Switzerland).

For testing the action on cell growth, cells were seeded into 96-well plates at the density of 5,000 cells/well, and after overnight standing, cells were treated with increasing concentrations of TNTs (1, 3, 10, and 30 μg/ml). After incubation for 72 hrs, 5 mg/ml MTT solution was added for another 4 hrs. The precipitated formazan crystals were solubilized in dimethyl sulfoxide, and the absorbance was measured at 545 nm using a microplate reader (SPECTROstarNano, BMG Labtech GmbH, Offenburg, Germany). Wells with untreated cells were utilized as control. The in vitro experiments were carried out twice with five parallels. TNTs were suspended in dimethylsulfoxide (DMSO), and the highest DMSO content of the medium (0.6%) did not substantially affect cell proliferation. Data were evaluated with GraphPad Prism version 10 for Windows software (GraphPad Software, San Diego, CA, USA), while statistical evaluation was performed with TIBCO Statistica v14.0.1.25 software (TIBCO Software Inc., Palo Alto, CA, USA).

4. RESULTS AND DISCUSSION

4.1. Determination of QTPP and CQAs

According to literature data, hydrothermally synthesized TNTs may be appropriate candidates to deliver APIs and to provide an efficient tool to be used in healthcare thanks to their good biocompatibility and small size, which is hard to be detected by the immune system. However, the identification of QTPP and related CQAs is an essential step in the development procedure of any product as they are application-related parameters which are unique for every case.

The properties of the manufactured nanotubes are dependent on the properties of the starting materials and on the operating conditions during hydrothermal reaction. Furthermore, they are also dependent on the conducted post-treatment steps.

In the present investigation, QTPP includes appropriate physical properties and stability; therapeutic effects; and appropriate PK, including absorption which follows drug release and ends with elimination, ensuring safe use for patients. Accordingly, morphology, which is unique in the case of

nanotubes, size, type, crystal structure, SSA, surface characteristics, yield of preparation, and drug loading were chosen as CQAs.

4.2. Risk assessment

To obtain TNTs with the required properties for a specific application, such as a pharmaceutical one, the hydrothermal synthesis method should be carefully optimized. As previously mentioned, in this chemical reaction, the starting precursor is subjected to high temperature in a concentrated alkaline medium for a specific duration, so the multiple parameters that are deeply involved in this procedure are highly responsible for the result. An Ishikawa diagram was proposed to summarize the necessary materials and processes for the utilization of TNTs as drug carriers starting from the moment of preparation until adjusting the surface properties by functionalization, loading with therapeutic molecules, and finally assessing the safety profile in order to incorporate these synthesized nanomaterials into final dosage form (oral, dermal, etc.).

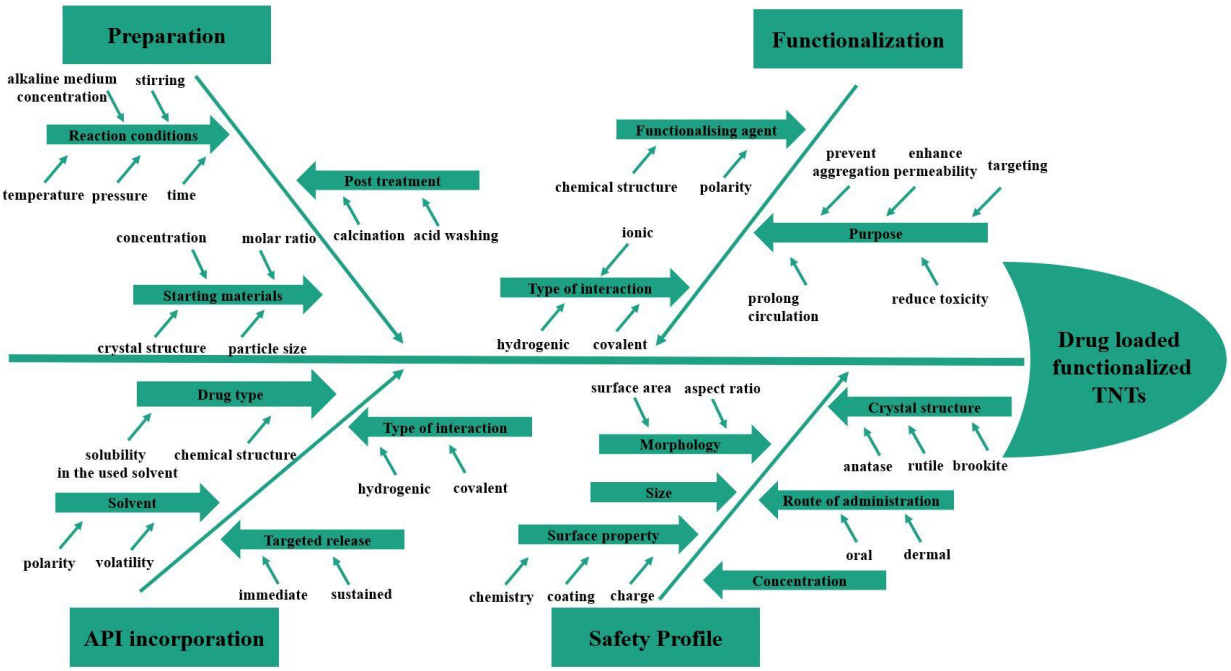

Figure 1. *Ishikawa diagram clarifying factors affecting the transformation procedure of TNTs into possible drug carriers*

Most of the selected CQAs are attributed to multiple elements of QTPP therefore a REM was created (Table 1) with the aid of LeanQbD v1.3.6. software to estimate the interdependence ratings between the collected elements. Furthermore, Figure 2. visualizes the priority of CQAs of hydrothermally synthesized TNTs as drug carriers depending on the chosen QTPP. According to Pareto analysis, the most significant CQAs were surface characteristics, morphology, SSA, size, type of TNT, crystal structure, drug loading, and yield.

Table 1. *Interdependence between CQAs and QTPP*

QTPP / CQA	Drug release	Stability	Safety profile	Therapeutic effect	PK	Physical attributes
Size	M	M	H	H	H	H
Crystal structure	L	H	H	L	L	M
Morphology	M	H	H	H	H	H
Specific surface area	H	M	H	H	H	H
Yield	L	L	L	L	L	L
TNTs type	L	H	H	L	M	H
Surface characteristics	H	H	H	H	H	M
Drug loading	L	L	L	H	L	M

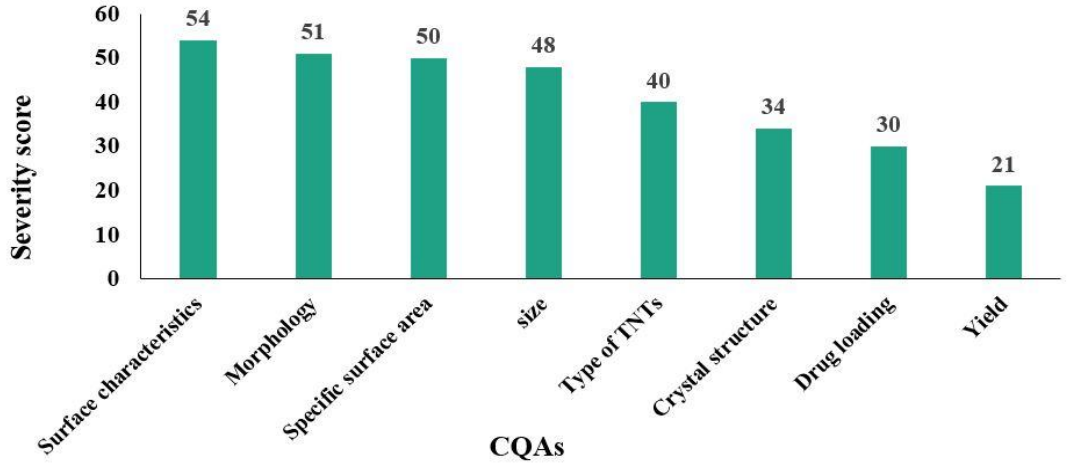

Figure 2. *Ranking of CQAs of hydrothermally synthesized TNTs as possible drug carriers*

The results of REM and Pareto analysis correspond well with the available data in literature, which repeatedly emphasized on the importance of tubular morphology and large surface area of TNTs in their possible usage as drug carriers due to the unique characteristics that come along with these two parameters, such as high cell internalization, as well as the ability to load APIs inside the tubular cavity or on their vast surface.

However, according to Pareto analysis, the properties of this vast surface, which can be controlled through modification with a proper molecule, are the most important factors as they can determine the success of the whole transformation procedure by their fundamental impact on the chosen QTPP. For example, surface modification could be used to control/enhance the release rate of loaded drugs and to enhance the PK properties (for example, absorption), which would have a huge influence on determining the used dosage form/route of administration and a high positive impact on the therapeutic effect. It could also be used to improve the safety and decrease toxicity. For example, surface functionalization using hydrophobic moieties could be a suitable way for enhancing the poor permeability of TNTs, which originates from their hydrophilic properties and hydroxylated surface. PEGylation could also present several advantages as it could be an appropriate solution for the aggregation problem in addition to making them undetectable by the immune system, prolonging their circulation time as well as reducing their toxicity. Determining the purpose of surface functionalization is an unavoidable step, as it would affect the type of molecule that is going to be fixed on the surface of TNTs and the type of interaction that will be created. This interaction is highly

dependent on the chemical structure of the functionalizing agent and the chemical bond that is going to be created, which is preferred to be a strong, long-lasting bond like covalent or ionic bonds rather than hydrogen ones. Hydrogen bonds could be more favorable in some cases, such as during API incorporation, as weak interaction between TNTs and APIs would not strongly affect the drug release unless this effect was the main target of incorporation. Drug incorporation is also considered to be a challenging step during the development of nanocarrier systems for drug delivery as multiple factors could affect the success of this procedure. Based on the authors' previous experience, the chemical properties of the used drug and solvents are essential during TNTs-drug composite formation. Solvent polarity (protic/aprotic nature) and volatility would highly affect the success of composite formation as they would affect the possibility of drug solubilization, the strength of solvent–drug interactions, and the ability to remove solvent from the system. In the ideal state, the requested drug–carrier interaction should be stronger than the one created between the drug molecules themselves or between the drug and the solvent. However, the strength/type of TNTs–drug interaction should be further investigated as it could be determined according to the desired release type (immediate or sustained). Finally, presenting titanate nanotubes as possible candidates as drug delivery systems is strongly dependent on their safety profile, which is still a matter of debate. However, several points have been thoroughly discussed in literature and could be considered as a starting point to build safer nanocarriers for therapeutic use. Morphology is one of these critical points affecting toxicity. It is true that the high surface area related to tubular morphology is considered as an advantage, but it also promotes cell penetration and the subsequent toxic effects. Surface chemistry/coating could also play a significant role in determining toxicity as changing the existing Na^+ ions on the surface of TNTs to H^+ or Mg^{2+} will increase their toxic effects, while applying a specific type of hydrophilic coating, such as PEGylation, could be a proper way to reduce aggregation and toxicity. The applied concentration is also crucial as increasing the used dose of these nanotubes would increase the impact of their hazard. In addition, the crystal structure could also affect the safety profile of TNTs as TiO_2 can exist in three different structures (anatase, rutile, and brookite), of which anatase appears to exert the highest toxic effects. Understanding these elements and their direct impact on toxicity would help researchers to optimize the preparation procedure and the subsequent steps to obtain a safer final product.

The same evaluation was performed again to study the importance and ranking of CPPs/CMAs during the transformation of hydrothermally synthesized TNTs into possible drug carriers (Table 2).

The most important CPPs were pH of the washing solution, reaction temperature, reaction time, calcination temperature, and stirring, while the important CMAs were precursor concentration, functionalizing agent, alkaline medium concentration, precursor particle size, precursor crystal structure, drug type, and used solvent (Fig. 3).

The results of this evaluation can be well justified with literature data as most of the performed studies reported that the washing step is an unavoidable phase in the preparation of TNTs, along with selection of the appropriate set of temperature, time and precursor concentration. The pH of washing solution is a significant factor in the washing process, which serves as the complementary step to obtain the desired tubular morphology of TNTs if the reaction conditions are not sufficient to achieve this. It would also have a major impact on most selected CQAs, such as the size of the resulted nanotubes, their type, SSA, and the preparation yield.

Table 2. *Interdependence between CQAs and CMAs/CPPs*

Process	**Preparation reaction**						
CPP/CMA / **CQA**	Temperature	Alkaline medium concent-ration	Time	Stirring	Precursor particle size	Precursor crystal structure	Precursor concentration
Size	H	L	H	M	L	L	H
Crystal structure	H	L	M	L	L	H	L
Morphology	H	H	H	M	H	H	H
SSA	H	H	H	L	H	L	H
Yield	H	H	H	H	L	L	H
TNTs type	L	L	L	L	L	L	L
Surface characteristics	L	L	L	L	L	L	L
Drug loading	L	L	L	L	L	L	L

Process	**Post treatment**		**Surface modification**	**API incorporation**	
CPP/CMA / **CQA**	pH of washing solution	Calcination temperature	Functionalizing agent	Drug type	Solvent
Size	H	L	M	L	L
Crystal structure	M	H	L	L	L
Morphology	H	H	L	L	L
SSA	H	H	L	L	L
Yield	H	H	L	L	L
TNTs type	H	L	L	L	L
Surface modification	L	L	H	M	L
Drug loading	L	L	M	H	H

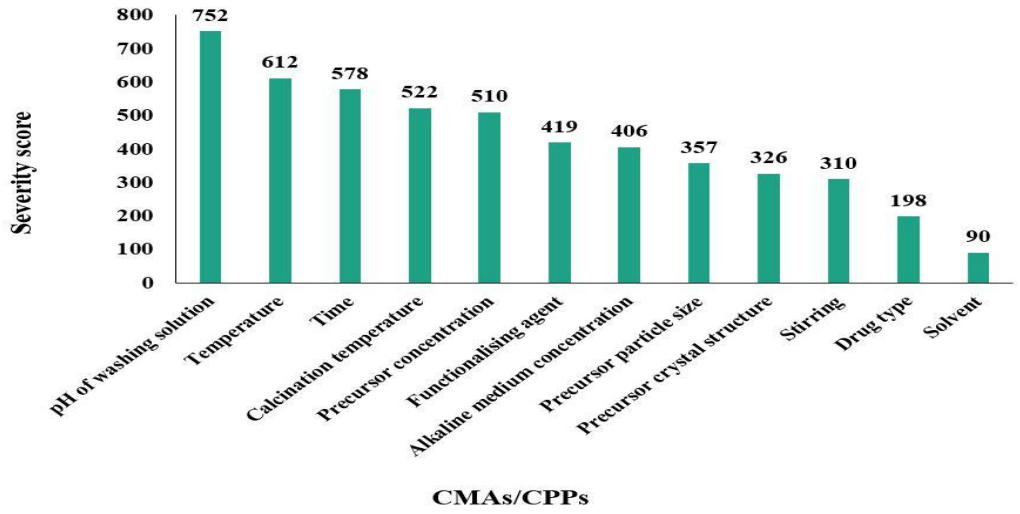

Figure 3. *Ranking of CMAs/CPPs during transformation of hydrothermally synthesized TNTs into possible drug carriers*

For these reasons, it is justified why pH of the washing solution is in the top ranking of CPPs, followed by reaction temperature and time, which also have a high impact on the majority of CQAs, like morphology, size, SSA, and yield. Moreover, these previously mentioned CQAs were influenced in the same way by the top-ranked CMA, which was precursor concentration. Again, these findings

correlated well with the data presented in literature, as the resulting top-ranked factors were the most discussed factors in literature, which supports their priority classification according to Pareto analysis results.

The detailed justification of the ranking results can be seen in the following paragraphs.

Temperature is the most discussed factor affecting the success of the preparation process as it serves as an energy supply to help the intermediate nanosheets to curl up and form nanotubes. Therefore, it should be used in the appropriate range, as too low temperature would not be enough to transform the starting nanoparticles/intermediate nanosheets to nanotubes. On the other hand, a too high temperature would also pose a problem, with several morphologies obtained other than the tubular one. This information could be of great importance if other morphologies are the morphologies of interest. In this case, the temperature can be raised up to create nanofibers (>150°C), nanorods or nanoribbons (>160°C), nanofibers or nanobelts (>170°C) and nanorods (>180°C). According to literature, the temperature range between 130 and 150°C is the most used in various research works, which represents a strong point of agreement and confirms the formation of complete nanotubes within this range. In addition, increasing the temperature within this range would increase the diameter, SSA, production yield, and enhance crystallinity without negatively affecting the requested tubular structure. Together, these specifications could be an advantage to be invested for the benefit of drug delivery systems.

Besides high temperature, TNTs preparation involves the usage of a highly concentrated alkaline medium, which supports the thermal energy to provide the required force for rolling up the sheet phase into a tubular one. Based on this, the concentration of this medium should also be within an appropriate range (10–15 M), as too low concentration would result in unreacted powder/untransformed sheets, while too high concentration would result in low yield. It is worth mentioning that this treatment should take sufficient time to give the intermediate phase an opportunity to transform into nanotubes and then to give the obtained nanotubes enough time to grow and elongate their length. Fifteen hours is probably the optimal time for the formation of pure nanotubes, with the possibility of increasing their length by increasing the treatment time to 24 hrs. However, caution should be taken as longer duration could lead to the formation of other morphologies, such as nanoribbons.

Furthermore, after a specific point, no length increase occurs, so further treatment will be just a waste of energy, which would subsequently have a negative impact on the economic cost.

The pressure inside the sealed autoclave during the reaction is one of the variable preparation conditions, but its impact on nanotube formation was not sufficiently discussed. Morgan et al. suggested that the pressure effect could be excluded from the significant factors affecting the formation of TNTs.

After the hydrothermal reaction is finished, the post-treatment processes (acid washing, calcination) take place and play a major role in preserving the desired morphology and obtaining the targeted characteristics. According to several publications, washing with acid could affect the size of TNTs and enhance their crystallinity. However, two critical factors were deeply discussed in literature regarding this process. Firstly, the pH of the washing solution was suggested to be kept between 2 and 4 to obtain a high yield and high SSA. The second one is the concentration of the used acid, on which there is a disagreement, as few studies recommended the use of low concentrations because high ones would destroy the tube-like morphology (>0.01 M) and result in the formation of granules

(>0.2 M) or clumps (>2 M), while others disagreed and indicated that high concentrations (0.5–1 M) could be the optimal range to use.
Another post-treatment process (calcination) can be used to enhance the crystallinity of the resulted materials, but caution should be taken not to negatively affect the morphology and thus the SSA.
The safest range to work in is between 200 and 400°C, as increasing temperature within this range would enhance crystallinity without negatively affecting the tubular structure. Higher temperature could lead to destroying the tubular structure (>450°C) and complete collapse of the nanotubes into irregular shapes (>500°C), nanorods (>540°C), nanoparticles (>600°C), or aggregates (>800°C).
It was noticed that the results discussed in literature were not always comparable as different operating conditions would lead to different and conflicted results. These differences make it difficult to establish strict guidelines for TNT preparation with prespecified characteristics. This means that studying the effect of different preparation factors separately poses a research challenge because they act as a full combination rather than independent ones. For example, a lower alkaline medium concentration could be compensated by the utilization of higher temperature. In the same context, lower temperature could be applied as long as prolonged treatment is used. Moreover, numerous factors during the preparation reaction could shorten the required duration for TNTs formation, such as stirring, smaller particle size of the starting material, or using less stable, higher energy anatase phase precursors. For this reason, the required parameters should be modified together to achieve the appropriate balance. This could be performed according to the requested final specifications of the prepared TNTs and according to the available laboratory instruments/equipment and the ranges within which they can work.
Careful evaluation and curation of the existing data enables us to better understand and model how to manipulate the operating conditions to achieve a specific purpose or affect a specific property of the resulted TNTs. For instance, increasing the factors (temperature, alkaline concentration, acid concentration) within the previously specified limits and decreasing the particle size of the starting precursor would increase SSA, but the opposite would happen by increasing the treatment time. This conclusion is very important because high surface area is one of the most significant characteristics of TNTs due to its direct impact on their possible functionalization and drug loading, so it could serve as an advantage for pharmaceutical applications as it successfully served earlier in energy saving applications and chemical catalysis.
Starting from a crystalline precursor (anatase) promotes the thermal stability of the resulted nanotubes, while washing them with acid will reduce the sodium content and resistance to temperature, resulting in H-TNTs that are thermally less stable than Na-TNTs.
The concentration of the used precursor also has an impact on the specifications of TNTs. High concentration increases the length/aspect ratio and diameter of the prepared TNTs but negatively affects the SSA. It could affect morphology; therefore, manipulating the Ti/Na molar ratio is an important step in shaping the structure of the resulted material into the desired direction (nanotubes, nanowires, nanofibers).

4.3. ANN modelling

As the risk assessment revealed complex relationships between the applicable CPPs, creating an ANN model to support the finding of optimal parameters was initiated. As discussed above, a dataset was created based on the existing literature to perform ANN modelling on the possible CQAs. The most discussed CQAs in literature were morphology and SSA of the obtained product, which enabled the

gathering of data sufficient to build an ANN-based model for the prediction of the possible outcome of a synthesis and post-treatment process. Furthermore, the global sensitivity analysis also enabled us to cross-validate the results obtained during the risk assessment procedure.

The structure (e.g., the percentages of rutile, anatase, and amorphous phases), surface area, and particle size of the starting precursor were considered as input CMAs, while the temperature and NaOH concentration of the reaction medium, the reaction time, the acid concentration of the washing liquid, and calcination temperature were selected as input CPPs. Most researchers used HCl in the washing step, but in some cases HNO_3 was applied.

The morphology of obtained products was described in literature with high versatility, which necessitated intensive data curation regarding this aspect. Besides the nanotubular morphology (1), the terms nanorods (2), nanofibers (3), and nanowires (4) were given to those products where one direction of particle growth was featured (products with an increasing length to diameter ratio) while the classification of particles where growth was featured in two directions were unified under the term nanoribbons (5). Spherical products were classified as nanoparticles (6) or spherical agglomerates (7). Moreover, two additional classes were made for those cases where no conversion (8) of the starting material was observed, or the obtained product was a mixture (9) of particles with different morphologies.

The best performing network for the classification of morphology had 10 input, 10 hidden, and 9 output neurons, where the activation of the hidden and output neurons were based on identity and softmax functions, respectively. The perfection of the classification for the training, testing, and validation subsets was 85.71%, 61.9%, and 76.19%, respectively. The results of the classification on the validation dataset are displayed in Figure 10**.** Most of the misclassified cases predicted the formation of nanotubes, which may have been due to over-representation of this class in the training subset, and a considerable increase in classification accuracy may be expected with a more balanced dataset. The results of the other retained networks showed high consistency with these results.

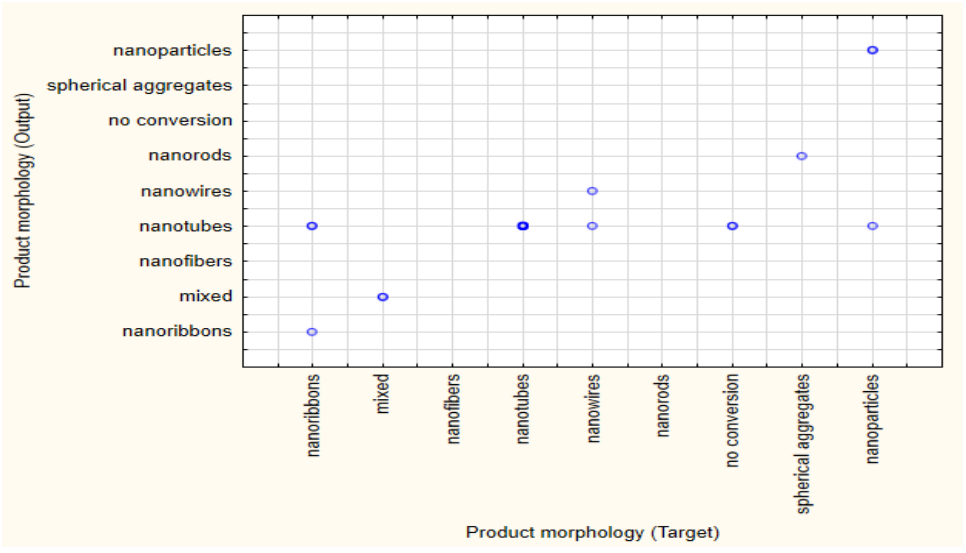

Figure 4. *Target vs. output results of the classification of particle morphology on the validation subset (light and dark blue dots represents one single case and multiple cases, respectively)*

The results of the global sensitivity analysis partially supported the results of the risk assessment, but the highest impact was exhibited by the calcination temperature, which was followed by particle size

and the structure of the precursor material (mostly by the amorphous content), with approximately equal contributions, while the NaOH content and the temperature of the reaction medium took third place, again with approximately equal contributions.
In the case of SSA modeling, the best performing network had 10 input, 10 hidden, and 1 output neurons, where the activation of the hidden and output neurons was based on tanh and logistic functions, respectively. The perfection of the classification for the training, testing, and validation subsets was 0.8924, 0.7834, and 0.9213, respectively. The target vs. output predictions on the validation subset are displayed in Figure 5.

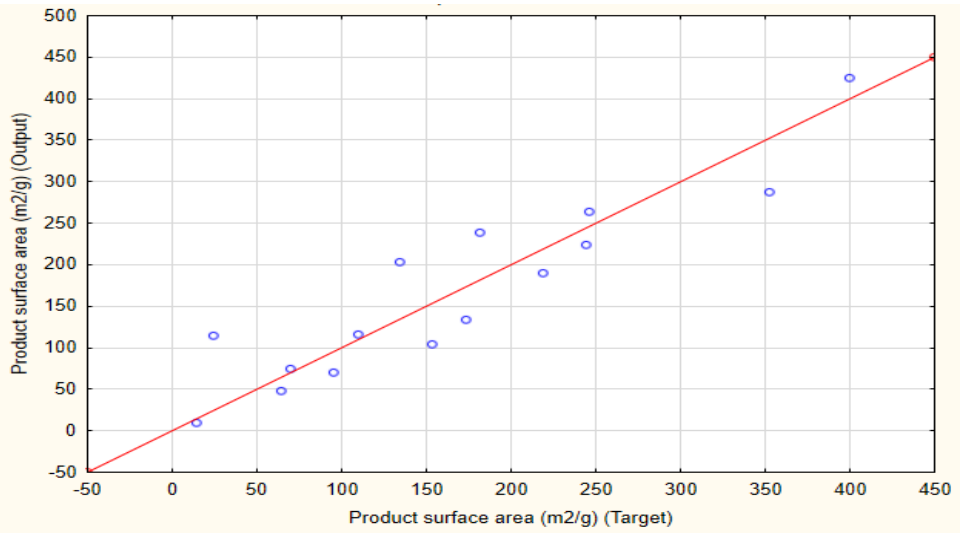

Figure 5. *Target vs. output results of SSA on the validation subset (red line represents the ideal target vs. output relation while blue dots represent the individual predictions)*

The global sensitivity analysis showed a similar picture as in the case of the classification of morphology. The most important factor affecting the SSA was found to be the calcination temperature, followed by the structure of the precursor materials (where also the amorphous content was the most predominant), the reaction temperature, and acid content of the washing liquid, respectively. The SSA of the starting precursor also plays a considerable role affecting the SSA of the product but, regarding the size of this effect, the different models exhibited some inconsistencies, which may be due to the fact that most of the available papers discussed limited information on the physical properties of the starting materials or the available data showed high versatility. One of the most used precursors was TiO_2 P25 (Degussa AG or Evonik AG, Germany), but its reported properties highly varied in literature. The specification datasheet refers to a unique anatase/rutile ratio, which varied between 80.21 ± 5.59% anatase and 17.40 ± 5.39% rutile contents, respectively, while some publications also reported 4.75 ± 5.72% amorphous content. Similarly, the average particle size of the anatase and rutile components varied between 10–48 nm and 14.4–51 nm, respectively. The SSA of the product was found to be 52.9 ± 12.08 m^2/g.

4.4. Synthesis of functionalized TNTs

Previous attempts to directly link PEG to TNTs (unpublished data) were not successful. Although PEG was successfully linked to TNTs via H-bonds, but the strength was not sufficient to hold the

complex together after dispersion in aqueous medium, so the PEG was rapidly detached from the surface of TNTs. Therefore, the main idea of creating a carboxylic arm on the surface of TNTs is to serve as a functional bridge and enable strong, covalent connecting of various molecules that could not be directly connected to TNTs hydroxylated surface.

4.4.1. Structural analysis of the products obtained

In the first attempt, the same method which was previously used for functionalization of TNTs with trichloro-octyl-silane was adopted to TCA. Nevertheless, this trial was not successful due to the fact that TCA has only one carboxyl group which was apparently not sufficient for creating a durable association with the surface of TNTs. In contrast, the functionalization experiments with citric acid appeared to be successful so an esterification attempt between the carboxylated surface of CA-TNTs with PEG was made depending on the theory that titanate could serve as a heterogenous catalyst for such chemical esterification reactions. For this reason, TNTs were used as a part of the reaction in addition to using them as a potential catalyst.

Unfortunately, this theory did not work, and the planned esterification reaction has failed as PEG was not bonded successfully to the carboxylated surface of TNTs, but it was probably removed with water during the washing step. According to these results, the choosing of multi-functional group carboxylic acid as citric acid is more favourable in term of using one group to interact with TNTs surface and leaving the other one or two for further interactions with additional molecules. Nevertheless, it also could be concluded that creating a weak interaction/association between the surface of TNTs and the carboxylic acids is not sufficient to step further for additional functionalization as the subsequent treatment could easily break this fragile association and result in bare TNTs after carboxylic acid being detached off the surface. This type of weak interactions such as electrostatic attractions and hydrogen bonds could be more favourable regarding TNTs loading with drugs so these drugs can be liberated later from this association and released to the biological medium. It is worth to mention that these interactions could slow the release rate of the drug or change its kinetic if they were sufficiently intense. On the other hand, stronger bonds are more favourable during functionalization/surface modification so this surface adjustment could be permanent to provide the TNTs with special specifications intended for special purposes. As the attempt for TNTs PEGylation via CA linker has failed, a third attempt using AA was applied where the hypothesis was to bond AA to TNTs by π-bonding via breaking up of its C=C bond. Although a weak interaction with the surface of TNTs was achieved similarly as the case with CA, the attempt was considered as a failed experiment. Since the characteristic peaks of AA (C=O stretching vibration at 1722 cm^{-1}, the OH bending at 1410 cm^{-1}, and the C-O stretching at 1299 cm^{-1}) exhibited significant shifting (1630, 1440 and 1277 cm^{-1}, respectively) in the spectrum of the reaction result, the C=C stretching vibration at 1636 cm^{-1} was covered while =C-H bending vibration at 986 cm^{-1} remained in an unchanged position (Fig. 6). These observations indicate that instead of the π-bonding with the C=C bond, the carboxylic group formed H-bond with the TNTs. In this case, the carboxyl group of acrylic acid would be occupied and thus would not be available to act as an extended arm for further functionalization. Moreover, as it was previously mentioned, such a weak interaction as H-bonds with acrylic acid is not enough to step further and use this acid as linker for additional molecules.

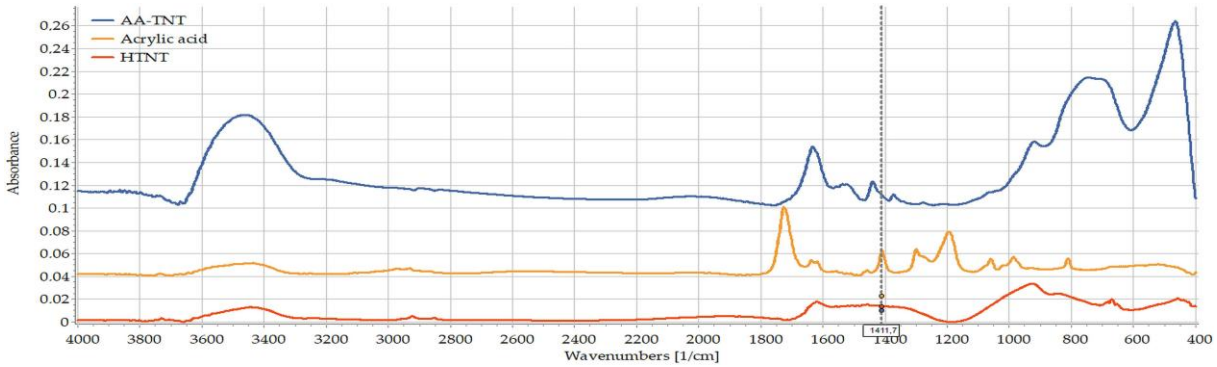

Figure 6. *FT-IR spectra of acrylic acid (yellow), TNTs (red) and AA-TNTs (blue)*

Based on the previous discussion, it is necessary to build a durable connection between the surface of TNTs and the carboxylic acid so it could tolerate the subsequent treatment for using it as a bridge connecting additional substances. For this reason, a reverse approach was conducted starting from bonding AA with the molecule of interest (PEG) as the initial step depending on the carbon double bond as the main site of interaction. Then, this synthesized adduct could be linked to the hydroxylated surface of TNTs. AA-PEG connection was done using free radical polymerization reaction which will present a new copolymer (PAA-PEG) with multiple carboxylic groups. These groups could be utilized later to connect more molecules with the carboxylic arm of the pegylated TNTs such as drugs, markers, or biological molecules.

Significant differences may be observed between the spectrum of the prepared copolymer and its precursors (PEG and AA) spectra (Fig. 7). The disappearance of the peak assigned to the vibration of H in C=C-H at 986 cm^{-1} is clear evidence of the polymerization reaction. Furthermore, the absorption peak of C-H stretching of methylene groups is also visible at 2928 cm^{-1} in the spectrum of the prepared copolymer and it could be a sign for the saturation of C=C bond in AA during the polymerization process. Regarding the C=O stretching range between 1600 and 1750 cm^{-1}, there were two peaks visible in the spectrum of AA assigned to C=C and C=O vibrations which are at 1636 and 1727 cm^{-1}, respectively. In the newly synthesized material, the peak at 1727 cm^{-1} is separated to 1715 and 1737 cm^{-1} indicating the presence of terminal and mid-chain carboxyl groups. The new peaks at 1558 and 1538 cm^{-1} may indicate that the carboxyl groups are presented in form of carboxylate anions. The slight decrement of the O-H bending vibration of PEG at 1349 cm^{-1} and the increasement of C-O stretching at 1245 cm^{-1} in the copolymer indicate that PEG is attached to the end of PAA chain through its terminal OH.

Regarding the spectrum of AA/PEG functionalized TNTs, the further shift of C=O stretching to 1706 cm^{-1}, and the multiple shifts of the peaks in the C-O stretching region at 1171, 1131 and 1038 cm^{-1} indicate that the copolymer is strongly attached to the surface of TNTs. A similar slight but considerable shift may be observed in the carboxylate stretching to 1560 and 1540 cm^{-1}. According to Pouran et al. and Liew et al., this shift indicates a strong ionic bond between the carboxylate anion and Ti ions which might be irreversible.

The results were cross confirmed by Raman spectroscopic investigations. It is well visible on Figure 8. that the intensive C=C stretching signal of AA at 1638 cm^{-1} is completely disappeared from the AA/PEG copolymer spectrum confirming the successful free radical polymerization reaction. The other characteristic peaks at 1452 cm^{-1}, 1726 cm^{-1} and 2931 cm^{-1} may be attributed to the stretching of carboxylate anion, carbonyl groups and CH_2 groups,

respectively. The peak at 2931 cm^{-1} may be clearly identified in the spectrum of AA/PEG functionalized TNTs in an unchanged position, but the other signals cannot be identified clearly due the fluorescence of TNTs.

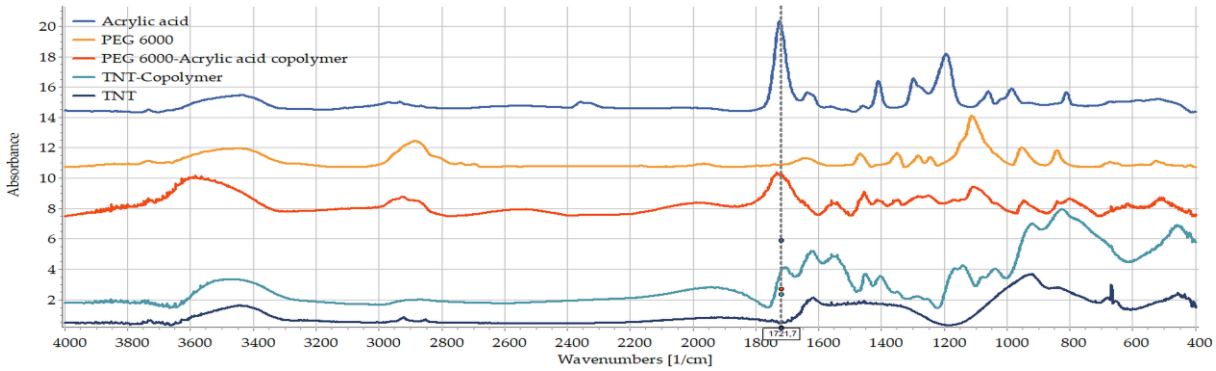

Figure 7. *FT-IR spectra of acrylic acid (blue), PEG 6000 (yellow), AA/PEG copolymer (red), TNT (dark blue) and the AA/PEG functionalized TNT (light blue)*

However, a new peak with small intensity has appeared in the spectrum at 1970 cm^{-1}, which may also indicate the presence of intermolecular interactions that may be due to the conjugated Ti-O-C vibrations.

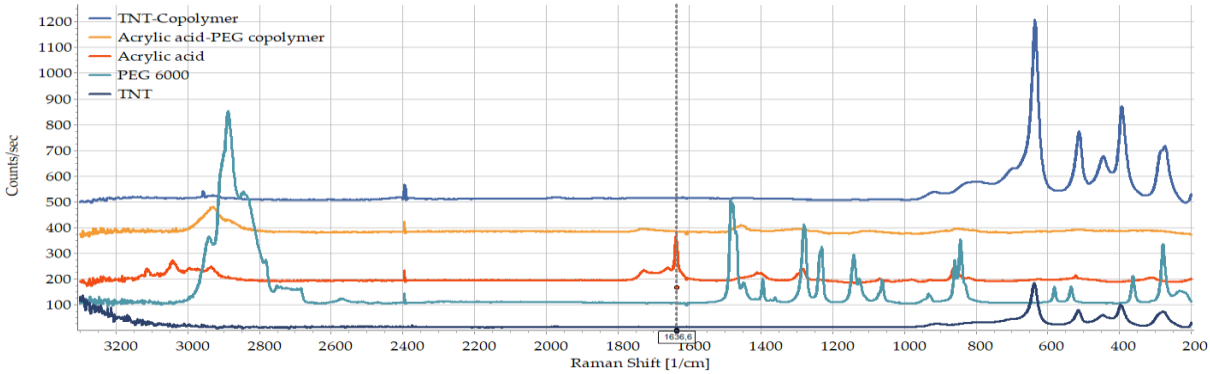

Figure 8. Raman spectra of acrylic acid (red), PEG 6000 (light blue), AA/PEG copolymer (yellow), TNT (dark blue) and the AA/PEG functionalized TNT (blue)

4.4.2. Morphology, size and surface charge investigations

Morphological investigations have also been conducted on the bare and functionalized samples, with both SEM and TEM. No considerable difference was observed between the bare and functionalized TNTs except of some fragmentation which may be due to the ultrasonication which was used to aid the dispersion of TNTs before the functionalization reaction. The diameter was found to be 9.56±1.56 nm vs. 10.42±2.03 nm, while the length was found to be 128.27±61,22 nm vs. 92.54±20,97 nm for bare vs. functionalized TNTs, respectively, according to TEM pictures. Nevertheless, these results show no correlation with those obtained with DLS measurements, where the hydrodynamic diameter in water was found to be 212.7± 0.2 nm vs. 417.2±10,1 nm for bare and functionalized TNTs, respectively. The larger average particle size in water in comparison with that in the dried state can be attributed to the existence of hydration layer that surrounds the hydrophilic nanotubes due to their hydroxylated surface, while the increased size of functionalized TNTs may be a further proof of the successful functionalization as the presence of AA/PEG copolymer on the surface of TNTs may lead

to greater size and increased interaction with water molecules probably due to their entrapment between its chains. The hydroxylated surface of TNTs provides a stable negative zeta potential (-36.47±0.35 mV) which corresponds well with the results of Bavykin et al. (-42.7 mV) and of Papa et al. who reported a strongly negative zeta potential above the isoelectric point (pH 3.6) of TNTs, and indicated that although these nanotubes would be negatively charged at the physiological pH (-34.5±4.3 mV), they can still interact well with the negatively charged cell surface achieving good internalisation supported by their tubular morphology. The functionalized TNTs exhibited the same stable negative zeta potential in purified water (-36,86±0,41 mV).

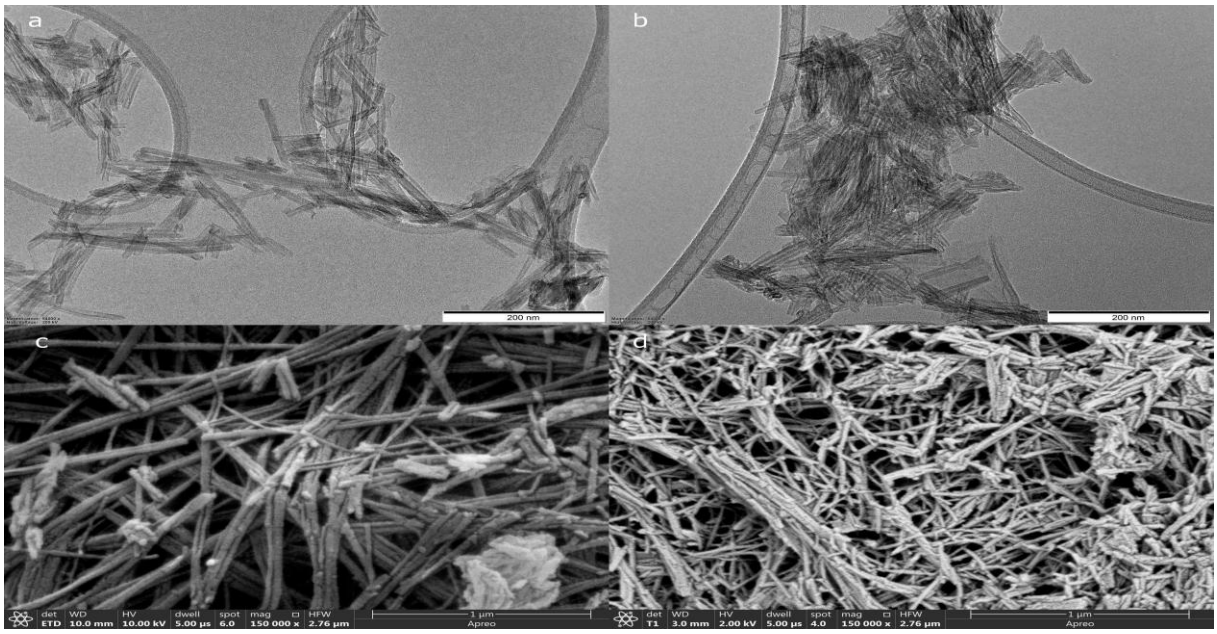

Figure 9. *TEM picture of bare (a) and AA/PEG functionalized (b) TNTs, SEM pictures of bare (c) and AA/PEG functionalized (d) TNTs*

However, there was a considerable difference in the behaviour of bare and functionalized TNTs in PBS buffer where despite of the unchanged zeta potential (-33.96±2.27 mV and -35.9±2.01, respectively), the bare TNTs exhibited visible precipitation which was in accordance with the size measurements that showed a size of 11037±7399 nm. According to our hypothesis, the PBS buffer induced a partial ion-exchange on the surface of TNTs which decreased the repulsion forces and induced the aggregation of nanotubes. In contrast, no similar effect was observed in the case of functionalized TNTs where only a slight increment was observed in the hydrodynamic diameter (570.43±26.53 nm), so functionalization here has a positive impact by reducing agglomeration and enhancing dispersibility and stability of TNTs in this medium.

Similar observations were detected in the PBS based cell culture medium where 11283±6480 nm and 837.83±18.48 nm size was detected for bare and functionalized TNTs, respectively. The increased size in the cell culture medium can be due to the decreased zeta potential (-3.14±1.79 mV and -23.2±0.95, for bare and functionalized TNTs, respectively) indicating strong TNTs-cell interactions. However, another possible explanation is that the presence of cells probably interferes with the measurement and might influence the results. Nevertheless, the smaller change in zeta potential of the functionalized samples in cell culture medium compared to that of the bare TNTs also supports the stabilizing effect of functionalization probably by sterically hindering the hydrophobic and

electrostatic interactions with the cells, reducing their adsorption on the surface, and probably simulating what could happen with the plasma components. However, decreasing the negative zeta potential would result in reducing the repulsion with cell membrane thus leading to the enhancement of cell permeation, higher penetration but also higher potential of toxic effects.

4.4.3. Cell viability study

The preliminary toxicity of the prepared TNTs has been investigated by a widely used viability assay against human adherent cancer cell lines and two different fibroblasts. The substances were applied in 1–30 μg/ml concentration range for 72 hrs. None of them exerted considerable cell growth inhibition as the growth inhibition values did not exceed 30% in any cases, so the investigated samples can be considered as safe (Fig. 10). The statistical analysis revealed no significant ($p>0.05$) difference between the tested samples. Regarding the sensitivity of various cell lines, the statistical evaluation confirmed that UPCI-SCC-154 oral squamous carcinoma cell line is the most sensitive cell line but in a non-concentration dependent manner. Typical concentration-dependent effects were observed only against UPCI-SCC-131 cells where the 30 μg/ml concentration exhibited significant increase in the growth inhibition in comparison with the 1 and 3 μg/ml concentrations ($p=0.012$ and $p=0.011$, respectively), but the inhibition did not exceeded the 30% level even in this case. The proliferation of ovarian cancer cell line A2780 and fibroblasts were practically not influenced by the treatment with TNTs. There were no significant ($p>0.05$) differences between human and murine fibroblasts (MRC5 and NIH/3T3, respectively) concerning the sensitivity toward the nanotubes. Though the presented cell-based viability results cannot substitute an appropriate toxicological study, they indicate that the tested substances have no relevant action on the cell growth and, therefore, no outstanding toxicity can be expected from later in vivo experiments.

These results agreed with the findings of Papa et al. who reported no cytotoxicity of TNTs on Chinese hamster ovary cell lines (CHO) after 24 hrs of exposure in a concentration up to 10 μg/ml. They were also in agreement with the findings of Fenyvesi et al. and Wadha et al. who demonstrated no cytotoxic effect of TNTs against Caco-2 cell lines in a concentration up to 5 mg/ml after short treatment (120 min) and against A549 epithelial cell lines in a concentration up to 1.1 mg/ml after long exposure (7 days), respectively. It is worth mentioning that these safety results were not limited to the bare TNTs, but the safety was also evidenced with different types of functionalization and with different chemical structures. For example, Ranjous et al. have reported the safety of silan-functionalized TNTs up to 1 mg/ml and stearate-functionalized TNTs up to 2 mg/ml on Caco-2 cell lines using MTT assay for a short exposure (120 mins). Papa et al. have also modified the surface of TNTs with polyethyleneimine (PEI) and then examined the toxicity with MTT assay on cardiomyocytes for 24 and 96 hrs. No significant toxicity was observed neither with TNTs nor with their functionalized samples in a concentration up to 10 μg/ml. These studies in addition to our research work are presented promising results to promote the future use of TNTs as safe nano carriers and novel platform for drug delivery.

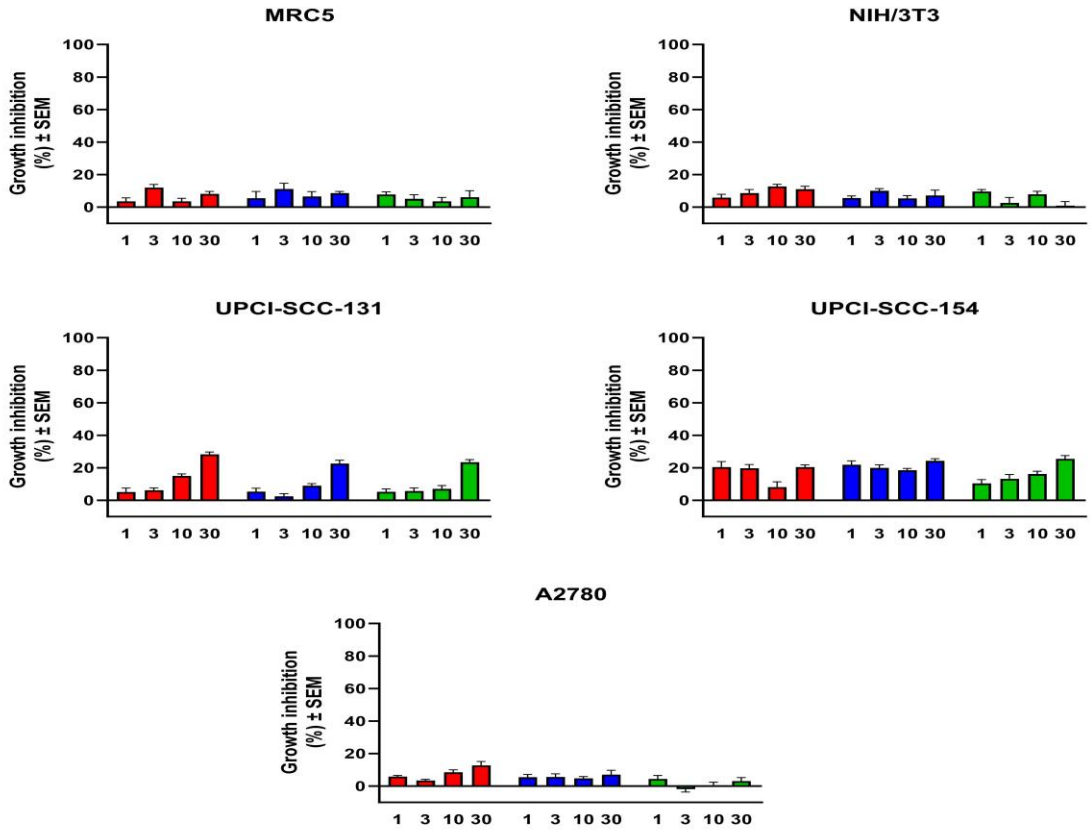

Figure 10. *Effects of the prepared nanotubes on the growth of the utilized fibroblasts (MRC5, NIH/3T3) and cancer cells. Numbers on horizontal axes indicate final concentrations in μg/ml, red, blue, and green columns mean TNT, AA/PEG copolymer, and AA/PEG functionalized TNT, respectively*

5. CONCLUSION

In this study, the transition of TNTs from their traditional chemical and industrial applications to pharmaceutical field has been proposed due to their high potentials and promising performance in several aspects of healthcare.

Hydrothermal treatment method was determined as the method of choice for the preparation of TNTs specified for pharmaceutical applications and for the subsequent investigation of their development to fit the pharmaceutical requirements.

This development procedure was proposed based on QbD approach and ANN modelling in order to establish a systematic guideline for researchers, surpassing the limitations of traditional methods of development.

Based on the obtained results, surface characteristics, morphology and SSA appeared to be the most important factors among the chosen CQAs. Only collected data of morphology and SSA were sufficient to build ANN models suitable to predict these properties with good perfection. The models confirmed that the previously applied synthesis route is proper to produce nanotubes with characteristics suitable for pharmaceutical applications.

The surface characteristics of the obtained nanotubes were further modified by preparing PEGylated TNTs due to its well-known advantages regarding preventing aggregation and reducing toxicity. According to our hypothesis, the successful PEGylation can be achieved by creating a carboxylic arm on the surface of TNTs. The type of the created interactions between the surface of TNTs and the

carboxylic arm, or between the carboxylic arm and PEG was also thoroughly discussed to determine the most appropriate type of interaction achieving the targeted mission. The original hypothesis was partially confirmed, since functionalization of TNTs with carboxyl groups were successfully achieved with multivalent carboxylic acids, but the obtained bond was not strong enough to survive the following esterification step. Therefore, a reverse approach should be applied to firstly create a carboxylic functionalized PEG molecule which could be then applied for the successful PEGylation of TNTs.

To the best of our knowledge, this is the first time to apply a combination of QbD tools and ANN modelling in the field of TNTs, aiming to produce a scientific-based recommendations and remarks for their transition into therapeutic practice.

6. NEW FINDINGS AND PRACTICAL RELEVANCE OF THE WORK

I. The transition of TNTs to the pharmaceutical field based on comprehensive screening of literature data will introduce promising drug nano carriers into therapeutic practice. Moreover, the implementation of QbD approach and ANN modelling in this transformation procedure will accelerate the process and build a scientific-based evidence for researchers to rely on.

II. Surface modification is considered as a key player in this transformation procedure due to its ability to manipulate the characteristics of the prepared nanomaterials to fit the requested mission therefore the created carboxylic arm on the surface of TNTs could facilitate further functionalisation and improvement of their properties/abilities to deliver drug molecules with maximal outcome and minimal adverse effects.

PUBLICATIONS AND PRESENTATIONS

PUBLICATIONS RELATED TO THE SUBJECT OF THE THESIS

1. **Saker, R.;** Jójárt-Laczkovich, O.; Regdon Jr, G.; Takács, T.; Szenti, I.; Bózsity-Faragó, N.; Zupkó, I.; Sovány, T. Surface Modification of Titanate Nanotubes with a Carboxylic Arm for Further Functionalization Intended to Pharmaceutical Applications, Pharmaceutics 2023, 15 (12), 2780
   doi: 10.3390/pharmaceutics15122780 IF =4.9 (2025)
2. **Saker, R.;** Hadi shammout; Regdon Jr, G.; Sovány, T. An Overview of Hydrothermally Synthesized Titanate Nanotubes: The Factors Affecting Preparation and Their Promising Pharmaceutical Applications.
   Pharmaceutics 2024, 16 (5), 635
   doi: 10.3390/pharmaceutics16050635 IF =4.9 (2025)
3. **Saker, R.**; Regdon Jr, G.; Sovány, T. Pharmacokinetics and toxicity of inorganic nanoparticles and the physicochemical properties/factors affecting them.
   Journal of Drug Delivery Science and Technology 2024, 99
   doi:10.1016/j.jddst.2024.105979 IF = 4.5 (2025)
4. **Saker, R.**; Ludasi, K.; Regdon Jr, G.; Sovány, T. (2025). Quality by Design-Based Methodology for Development of Titanate Nanotubes Specified for Pharmaceutical Applications Based on Risk Assessment and Artificial Neural Network Modeling. Pharmaceutics 2025, 17(1), 47
   doi: 10.3390/pharmaceutics17010047 IF =4.9 (2025)

PUBLICATIONS NOT RELATED TO THE SUBJECT OF THE THESIS

1. Shammou H; **Saker, R.;** Ludasi, K.; Sovány, T. An Overview of selective laser sintering technology: principles, formulations, applications and promising potentials in pharmaceutics. Journal of Drug Delivery Science and Technology 2025, 111
   Doi: 10.1016/j.jddst.2025.107166 IF = 4.5 (2025)

PRESENTATIONS

Verbal presentations:

1. **Ranim Saker,** Géza Regdon jr., Tamás Sovány: Preparation of functionalized titanate nanotubes to improve toxicological profile and bioavailability. IV. Symposium of Young Researchers on Pharmaceutical Technology, Biotechnology and Regulatory Science. January 20, 2021. Szeged, Hungary.
2. Tamás Sovány**, Ranim Saker,** Yasmin Ranjous, Géza Regdon jr.,: Functionalization and application possibilities of titanate nanotubes for drug delivery. 22nd IMEK conference. July 15, 2022. Szeged, Hungary.
3. **Ranim Saker,** Géza Regdon jr., Tamás Sovány: Investigation of carboxylic acids possible application as linkers on the surface of titanate nanotubes for further functionalisation. V.

Symposium of Young Researchers on Pharmaceutical Technology, Biotechnology and Regulatory Science. January 19, 2023. Szeged, Hungary.

4. Tamás Sovány**, Ranim Saker,** Géza Regdon jr.,: Titanate nanotubes as potential platforms for drug delivery. 39th Technological Days. September 7, 2023. Bratislava, Slovakia.
5. **Ranim Saker,** Géza Regdon jr., Tamás Sovány: The application of carboxylic acids on the surface of titanate nanotubes for further functionalization. 14th Central European Symposium on Pharmaceutical Technology. September 30, 2023. Ohrid, North Macedonia.
6. **Ranim Saker:** Carboxylic acids as linkers on the surface of titanate nanotubes for further functionalization. Clauder otto competition. November 17, 2023. Budapest, Hungary.
7. **Ranim Saker,** Géza Regdon jr., Tamás Sovány: Carboxylic acids as linkers on the surface of titanate nanotubes for further functionalization. VI. Symposium of Young Researchers on Pharmaceutical Technology, Biotechnology and Regulatory Science. January 25, 2021. Szeged, Hungary.
8. **Ranim Saker,** Géza Regdon jr., Tamás Sovány: QbD Guided Approach for the Development of Titanate Nanotubes Specified for Pharmaceutical Applications. VII. Symposium of Young Researchers on Pharmaceutical Technology, Biotechnology and Regulatory Science. January 29, 2025. Szeged, Hungary.

Poster presentations:

1. Tamás Sovány, Dalma Erdei, **Ranim Saker,** Yasmin Ranjous, Géza Regdon jr.,: Preparation and Examination of the compressibility of titanate nanotube-API composites. 10th International Granulation Workshop. June 21, 2023. Sheffield, England.
2. **Ranim Saker**, Orsolya Jójárt-Laczkovich, Géza Regdon, Jr., Tamás Takács, Imre Szenti, Noémi Bózsity-Faragó, István Zupkó, and Tamás Sovány,: Indirect PEGylation of titanate nanotubes with the aid of carboxylic acids as surface linkers. 27th Spring Wind Conference. May 3-5, 2024, Budapest, Hungary.
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