Formulation, Characterization, and Evaluation of Ticagrelor-loaded Nano Micelles Enhance Intestinal Absorption

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ABSTRACT

The biopharmaceutical categorization system (BCS) assigns ticagrelor as a class IV medication because of its limited solubility, permeability, and low bioavailability (36%). It was fabricated into nano micelles to adopt the absorption issue with ticagrelor. To improve ticagrelor properties related to poor solubility and permeability, it is combined with D-alpha-Tocopherol Polyethene Glycol 1000 Succinate (TPGS), As TPGS has solubilizing action, a protective effect against cytochrome P450 3A4 enzyme and a permeation enhancer by suppressing the p-glycoprotein efflux transporter. Therefore, it was chosen for the preparation of ticagrelor Nano micelles.

These micelles were created using a straightforward solvent casting technique. A 1:2 (Ticagrelor: TPGS) ratio and 10mL water proved to be the most appropriate formula in the trial design, which comprised three variables of ethanol, water, and TPGS contents. The gathered information disclosed that the encapsulation effectiveness was 98%, with a spherical form with 42nm diameter, a polydispersity value of 0.287 and a homogenous.

Keywords: Ticagrelor, TPGS, Nano micelles, Permeability enhancement, Ex vivo diffusion model.

INTRODUCTION

Oral administration is the preferred medicine delivery method because it is noninvasive, has excellent patient compliance, is cheap, and allows various dosage forms to be created. Oral drugs account for approximately 90% of commercially available formulas for managing gastrointestinal tract (GIT). disease or systemic effects. In contrast to other routes, oral medications must successfully pass through the various biological barriers in the GIT to reach systemic circulation[1,2]. Some of these barriers are the environment's changing pH, the existence of an enzyme that can cause medication breakdown as protein-based formulations, the impact of food, and GIT diseases[3]. Regarding intestinal permeability, drugs given orally should penetrate through the intestinal membrane, acting as the principal cellular barrier for drug absorption into the systemic circulation[4]. As a result, both the solubility of the medicine in intestinal fluid and the permeability through the membrane significantly influence the amount of drug absorption[5]. One of the most effective strategies for overcoming intestinal dissolution and absorption issues is the nano-sized formulation, like nano micelles[6].

Nano Micelles are self-aggregated with core and shell-like shapes and particle sizes ranging from 10 to 100 nanometers. They are produced from substances with amphiphilic properties as surfactants and polymers. The head of surfactants may be ionic, non-ionic or zwitterionic. The polymeric micelles are produced from block co-polymer as polyethylene glycol[7]. The difference between the surfactant and polymeric micelles is that the former is generated at higher critical micelles concentration and has larger particle size and lesser stability due to the units within the structure of a polymer are linked to each other by covalent bonds to form the final structure of a polymer with amphiphilic properties, this interaction does not occur between surfactant molecules[8].

The solvent system controls these micelles' orientation in an aqueous medium, and the hydrophilic portion will orient the solvent, forming an outer layer through hydrogen bonding. And the hydrophobic component will create an isolated core region through van der Waals bonds[9]. Solvent casting is one of the procedures for making Nano micelles. It involves using an organic solvent like ethanol to dissolve polymer and drug, letting the ethanol evaporate to make the thin film, and then rehydrating it with water[10].

Ticagrelor (TCG) is a present-day antithrombotic medication. It reversibly binds to adenosine diphosphate (ADP) receptors found on platelet cells. Patients with myocardial revascularization require this activity to protect the heart tissue from an elevated plasma adenosine concentration, increasing blood flow to the heart and lowering the risk of embolism[11]. It is recommended as an alternative to clopidogrel in managing acute coronary syndrome[12]. Regarding safety, treatment with clopidogrel is similar to TCG[13]. Chemically, Ticagrelor is a cyclopentyl-triazole-pyrimidine. Due to its deficient

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solubility and poor permeation, it is categorized as a Class IV of the biopharmaceutics categorization system (BCS), exhibiting about 36% oral bioavailability[14]. Oral drug delivery has gotten the most attention due to patient compliance, convenience, and high safety of the oral route[15]. Improving oral absorption for medications falling under BCS class IV is challenging because the formulator should consider the drug's weak water solubility, individual variability, food interaction, and poor penetration. Additionally, most of these medications function as substrates for p-glycoprotein and metabolic enzymes[16]. Therefore, it is required to formulate ticagrelor with a solubility and permeability enhancement strategy.

The structural resemblance of ticagrelor to adenosine triphosphate (ATP) results in its pharmacological activity[17]. This resembles the p-glycoprotein substrate's structure[18]. As demonstrated above, ticagrelor has poor solubility in water and poor permeability, leading to low oral bioavailability. To overcome these problems, we need excipients to have the ability for enhanced oral absorption of TCG, like TPGS. Ticagrelor has developed in recent research as nanosuspension[19], Lipid nanocarriers with patterned surfaces[14] and a medication delivery method that self-emulsifies[20]. Still, there is no research for developing ticagrelor as nano micelles, so we believe preparing TCG as nano micelles containing a suppressor of p-glycoprotein efflux transporter like D-alpha-Tocopherol Polyethene Glycol 1000 Succinate (TPGS) could improve intestinal permeation.

TPGS is a non-ionic surfactant created by combining vitamin E with polyethylene glycol 1000, which has amphiphilic characteristics and can form stable micelles in water at concentrations (CMC) as low as 0.02wt%. It has a hydrophilic/lipophilic balance value of about 13.2, a molecular weight of 1513 and a melting point of about 37-41 ceils. TPGS has solubilizing action on compounds that are practically poor soluble in water. The protective effect of TPGS against cytochrome P450 3A4 enzyme (the principal enzyme responsible for drug metabolism inside the intestines) might have to enhancement in oral absorption of medicine acts as a substrate for this enzyme[21]. TPGS can be used as a stabilizer, emulsifier and co-polymer for the enhancement delivery of anticancer drugs[22].

Furthermore, because a polyethylene glycol chain is included in its structure, TPGS has other benefits, such as safety, avoiding reticuloendothelial recognition, biocompatibility, and biodegradability[10]. Also, TPGS serve as a permeation enhancer by suppressing the p-glycoprotein efflux transporter in the internal lining of the intestine because this transporter can eject drug molecules rearward to the lumen of the intestine. One of the approaches to improve intestinal absorption is combining with substances that act as an inhibitor for p-glycoprotein transporters as drugs belonging to BCS class IV, such as etoposide and aripiprazole[18]. Additionally, TPGS can be used in a wide range of Nano formulation as micelles, liposomes, nanoemulsions, nanoparticles and Nanocrystals[23].

Identifying the causes of poor oral absorption is critical because a suitable excipient can be included within the formulation to solve limited oral absorption challenges[24].

Aim of the works: To formulate the TCG-loaded nanomicelles containing TPGS to improve both the dissolution and the absorption of TCG[26].

MATERIALS AND METHOD

Materials: Ticagrelor was bought from Anhui Hailkan pharmaceutical co, Ltd – china; Vitamin E TPGS was bought from Baoji Guokang Bio-technology co. Ltd in China, other reagents were bought (formerly Sigma Aldrich).

Method: Preparation of ticagrelor-loaded nano micelles: Ticagrelor-loaded nano micelles were prepared by a modified solvent casting. By forming a homogenous solution of drug and surfactant with aid of organic solvent followed solvent evaporation to thin film formation on high vacuum and self-assembling to nanomicelles on rehydration[26-28]. The Minitab® (version 21)[29-44] was used to design the experiments to prepare 27 formulas using three variable factors at three levels of full factorial designs, including the amount of TPGS, the water, and ethanol volumes, according to Table 1. The medication was combined with a predetermined amount of TPGS. With heating to 37° C to melt TPGS and make the drug more wetted with TPGS, the formula was supplemented with ethanol. After solubilization, the solvent was completely vaporized with rotary evaporator. Once a thin layer was formed, for hydration reasons, varying quantities of deionized water were included with agitation on a magnetic stirrer for 10minutes at 500rpm and 25° C, then left for resting, and a homogenous mixture was formed[9,45,46]. The results of the experiment design have been assessed using three factors designed on Minitab software: drug: TPGS weight ratio, solvent volume, and aqueous phase volume. According to the analysis of the factorial design of results based on obtained spherical diameters of the prepared formulas, a new design would be developed to prepare TCG nanomicelles with possible smallest diameter with good PDI.

Table 1: Composition of ticagrelor nano micelle formulas

Formulation	Drug (mg)	TPGS (mg)	Ethanol(mL)	Water (mL)
F1	30	60	0.75	5
F2	30	90	0.75	5
F3	30	135	0.75	5
F4	30	60	1.5	5
F5	30	90	1.5	5
F6	30	135	1.5	5
F7	30	60	6	5
F8	30	90	6	5
F9	30	135	6	5
F10	30	60	0.75	10
F11	30	90	0.75	10
F12	30	135	0.75	10
F13	30	60	1.5	10
F14	30	90	1.5	10
F15	30	135	1.5	10
F16	30	60	6	10
F17	30	90	6	10
F18	30	135	6	10
F19	30	60	0.75	15
F20	30	90	0.75	15
F21	30	135	0.75	15
F22	30	60	1.5	15
F23	30	90	1.5	15
F24	30	135	1.5	15
F25	30	60	6	15
F26	30	90	6	15
F27	30	135	6	15

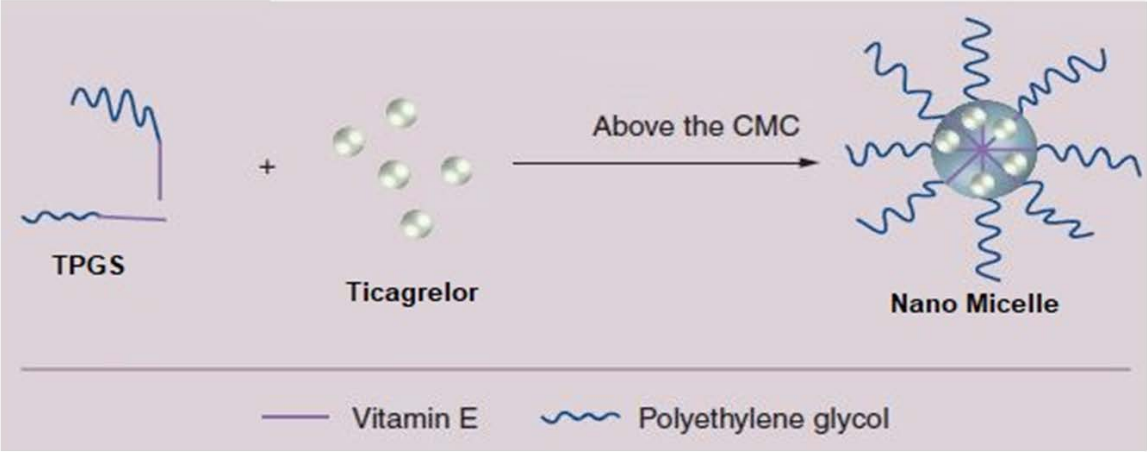

Figure 1: The formation of ticagrelor nano micelle adopted[25]

Effect of sonication: As previously mentioned, the 27 formulas of Ticagrelor nano micelles listed in Table 1 were prepared again. Afterwards, preparations were rested for 3min and exposed to ultrasound probe sonication (BIOBASE - China) at 300Watts, 3sec on, and 3sec off at 25° C for 30 seconds. Using DLS equipment, samples were analyzed to determine particle size and polydispersity index (PDI)[47,48]. The formula with lowest spherical diameter and good PDI to be selected for further evaluations and permeation study.

Characterization of ticagrelor-loaded nano micelles selected formula:

Spherical diameter, Polydispersity index (PDI) and surface charge analysis: Particle size and PDI analysis might be utilized to explore the physical characteristics of ticagrelor Nano micelles. This analysis employed dynamic light scattering (Malvern Instruments, Zetasizer NanoZS, UK)[49,50]. Particle size and PDI were determined at 25° C and a 173° angle using a 1cm polystyrene dip cell[51].

Entrapment Efficiency (EE%) and Drug Loading (DL) of ticagrelor in nano micelles: The dialysis centrifugation method has determined the entrapment efficiency for ticagrelor nano micelles. The technique was made by placing 2mL of formula in an 8000-14000 molecular weight cutoff dialysis membrane and tidily fixed as a bag on the top with a cup of the of a centrifuge tube, and on centrifugation for 45minute at 6000rpm, and 25° C. The membrane acts as a filter membrane, and then free drugs and drugs contained within micelles were analyzed using a UV spectrophotometer (Cecil Instruments – England). Then the entrapment efficiency and the drug loading were computed using the equations shown below[52]:

$$EE\% = \frac{weight\ of\ starting\ drug\ use\ -the\ weight\ of\ free\ drug}{total\ Weight\ of\ drug} \times 100\%$$

$$DL\% = \frac{weight\ of\ the\ drug\ in\ nano\ micelles\ formula}{Weight\ of\ the\ drug\ and\ surfactant} \times 100\%$$

Differential Scanning Calorimetric studies (DSC): This test was made (using DSC named Shimadzu DSC-60 from Japan) for ticagrelor, TPGS, physical mixture, and ticagrelor Nano micelles were loaded in an aluminium cell, and a test was carried out to obtain DSC spectral data at nitrogen flow and 20 degrees Celsius per minute[45].

TEM Imaging: Transmission electron microscope (TEM) images were taken to analyze the morphological properties of ticagrelor Nano micelles. They were initially put onto 200-mesh-coated copper grids, then dehydrated at 60°C, and this TEM model proposed Supra-55VP Zeiss (Germany)[53].

Study on intestinal permeability under ex vivo conditions: This study used a newly developed ex-vivo apparatus and methodology[54]. The test was made for raw materials in an aqueous mixture and ticagrelor nano micelles to assess intestinal motility and permeability in the same experiment[39]. That relied on a segment extracted from an intestinal rat male[19]. The rat was anesthetized after fasting for 12h. The abdomen opened at the midline position, then the gut was extracted and washed with glucose saline, and the intestinal segment (about 5cm in length) was removed from the jejunum site. Ligation has been made carefully to the lower end of this segment with a silk suture and a long silk thread attached to the top end of the displacement transducer-475 with an iworx system made by DOVER NH in the USA[40]. This segment was vertically inserted in a tissue chamber filled with Tyrode solution to drive a volume of 30mL. The solution was supplied with 95% O_2 and 5% CO_2 at 37° C and pH 7.4 to provide similar conditions to the intestine in humans. The evaluation of micelle behavior following contact with a segment and disassembly in the bio-relevant fluid was carried out during this experiment. DLS was applied to determine whether it provided evidence of drug release from micelles[55]. To start the TCG permeation test, 0.25mL of raw TCG aqueous mixture or selected nano micelles formula was loaded into the segment. A 2mL was withdrawn at specific scheduled times for analyzing the diffused drug from an intestinal segment by a UV spectrophotometer[56,57]. To ensure accuracy, a triplicate of this test was performed. The kinetic of drug diffusion was measured using the first line equation (equation-1) of drug concentration diffused versus time.

Y = Slope. X + Intercept Equation no. …….(1)

Stability study

Storage stability: To test for stability, the selected formula tested at various time intervals for homogeneity, particle size, and polydispersity index[30,58].

pH stability study: This study was made for selected formula by a 0.5mL addition to 30mL of each 0.1N HCl (pH 1.2) and phosphate buffer (pH 7.4) to achieve 60-fold dilution. Then monitored by visual investigation, the occurrence of Tyndall phenomena using red laser light and DLS for particle size and PDI analysis at 0, 2, 4, 8, and 24h at 37° C. the experiment worked out in triplicate[58].

Dilution stability: The dilution test was proposed to determine if a significant amount of the external (continuous) phase could be

introduced to the chosen nano-micelle formula without generating stability issues. This test was made by adding 0.5mL of selected formula to 3mL (1:6) and 6mL (1:12) of distilled water to achieve a level of dilution that the concentration of TPGs was equal to 1mg/mL and 0.5mg/mL; respectively, this addition was made directly after the formula preparation. After resting for one hour, these formulas were examined visually and measured particle size and PDI; the experiment was done in triplicate[41].

Statistical analysis: The General Factorial regression on Minitab software (version21) was used to analyze the factorial design responses at confidence interval of 0.95. The statistical analysis utilizing ANOVA "analysis of variance" at p-value (≤0.05) was applied to analyze the effect of sonication on the spherical diameters and PDI of the prepared formulas[59].

RESULTS AND DISCUSSIONS

Preparation of ticagrelor nano micelles: The results exhibited all the designed formulas were successfully prepared with non-sized micelles, as in Figures 2 and 3, with a maximum average spherical diameter of 126.6nm at F24 and a minimum size of 42nm at F4. The F4 was selected for further studies since smaller micelles can penetrate and absorb through the paracellular and transcellular pathways and enhance intestinal absorption[14]. PDI values (0.2-0.4) are acceptable for narrow size distribution. Also, the smaller PDI value indicated more homogeneity and more formulation stability. TPGS was chosen for the formulation of ticagrelor nano micelles because its capability to act as surface active agent and to suppress the pGP for overcoming undesirable drug properties related to poor oral absorption. These properties include: Vitamin E TPGS has a lower CMC of about 0.02 percent, which provides high formulation stability. This characteristic is critical in ticagrelor nano micelles. In addition, Increased water dissolution of inadequately soluble medicines and upgraded oral absorption for BCS class IV medications like ticagrelor would be attained with TPGS[21]. We used ethanol, a suitable solvent for solubilizing the drug and the TPGS and stirring to develop the formation of a homogeneous mixture.

Using experimental design on Minitab reveals that water volume is the most important element in determining particle size. The minor influencing factor is the volume of ethanol, based on measurements of particle size produced from the experiment design shown in Table 2. The analysis of variance showed that regarding the importance, hydration comes out on top., with the lowest p-value of about 0.018, according to figure 6, as illustrated. Based on these results, we made seven other formulations with a lower weight of TPGS and a lower volume of water and ethanol, as shown in Table 3. Then after the measurement of particle size and PDI, we found that the lower particle size produced from the experiment design was about 42 nm, while the lower particle size produced from the optimization design is about 60nm. Therefore, from the experiment design, we choose the three formulas with the particle size, PDI minimization, and highest stability, F4, F15, and F19, to measure further characterization for these selected formulas.

Table 2: Analysis of variance on general factorial regression: Spherical diameters of nano micelles versus TPGS; ethanol; water

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	6	4493.1	748.9	2.09	0.100
Linear	6	4493.1	748.9	2.09	0.100
TPGS	2	718.5	359.2	1.00	0.384
ETH	2	210.4	105.2	0.29	0.748
H2O	2	3564.2	1782.1	4.99	0.018
Error	20	7149.7	357.5		
Total	26	11642.8			

Table 3: Ticagrelor Nano micelles prepared depending on the outcomes from optimization design

Formu-lation	Drug (mg)	TPGS (mg)	Ethanol (ml)	Water (ml)	Z- average (nm)	PDI
F27	30	60	0.75	5	60.75	0.405
F28	30	60	0.75	4	60.75	0.447
F29	30	60	0.75	3	62.97	0.445
F30	30	45	0.75	5	138	0.299
F31	30	30	0.75	5	74.86	0.441
F32	30	60	0.5	5	66.98	0.299
F33	30	60	0.25	5	60.18	0.374

Sonication effect: The result in figures 4 and 5 explain the sonication effect on the spherical diameter and PDI of prepared formulas. Sonication showed no significant effect on both parameters. However, it gave a minimum particle size of 52nm in the F4 formula, whereas stirring alone gives a particle size of 42 nm for a recipe with identical content, the study of polymeric micelles prepared by sonication only show that; sonication can decrease the particle size from 800nm to 200nm and from 600nm to 100nm after 10 minutes, but it cannot reduce particle size smaller than 100nm with an increased sonication time to 60 minutes[46].

Characterization of ticagrelor-loaded Nano micelles selected formula:

Spherical diameter, Polydispersity index (PDI) and surface charge analysis: The smaller particle size helps improve penetration through the biological membrane[60]. From figure 2, F4 had a smaller spherical diameter of 42nm and a lower PDI value of 0.2 (which is acceptable to give a narrow distribution size of the particle); therefore, it's the most optimum formula; many samples had PDI values close to 0.4. Lower PDI values indicate a tight particle size distribution in the produced micelles, within the acceptable range of 0.05–0.7 for preferred particle distribution[61]. To ensure the stability of the preparations, the zeta potential was analyzed for a selected formula that was found to be -13. This value revealed that the F4 has less affinity to aggregation[62].

Entrapment efficacy % (EE) and Drug Loading% (DL): TCG. was successfully entrapped within the selected formula as demonstrated by the per cent EE and per cent DL findings in Table 3, that the proportion of EE is between 90% and 98%. It was reported that TPGS micelles with hydrophobic drug provide entrapment efficiency with high per cent 99%,97%,92%[29,63,64] and that this entrapment is caused by hydrogen bonding and Vander vales forces and that as the concentration of TPGS rises, so does the drug loading[29], the perfect formula with high entrapment about 98%is F4.

Table 4: Percentage of entrapment efficiency and drug loading of ticagrelor nano micelles formulas (mean SD, n=3)

F	Entrapment Efficiency %	Drug loading %
F4	98 ± 0.06	21 ±1.42
F19	90 ± 1.8	26 ± 1.43
F15	96 ± 0.45	23 ± 1.59

Differential Scanning Calorimetric studies (DSC)

To identify the drug's thermal properties, a DSC examination of the drug and its excipient is necessary to determine whether it is in an amorphous or crystalline state after formulation[65,66]. Ticagrelor and TPGS had melting points at 140° C and 37° C[67,68], respectively. These peaks are present in figure 7 according to the DSC thermogram of raw TCG., raw TPGS, and a physical mixture of TCG and TPGS.

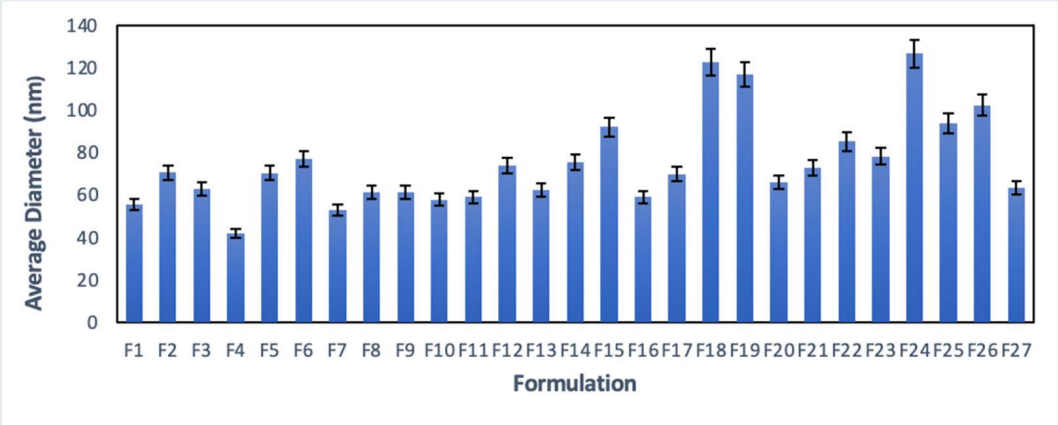

Figure 2: Average spherical diameter (n=3) for prepared ticagrelor-loaded nano micelles

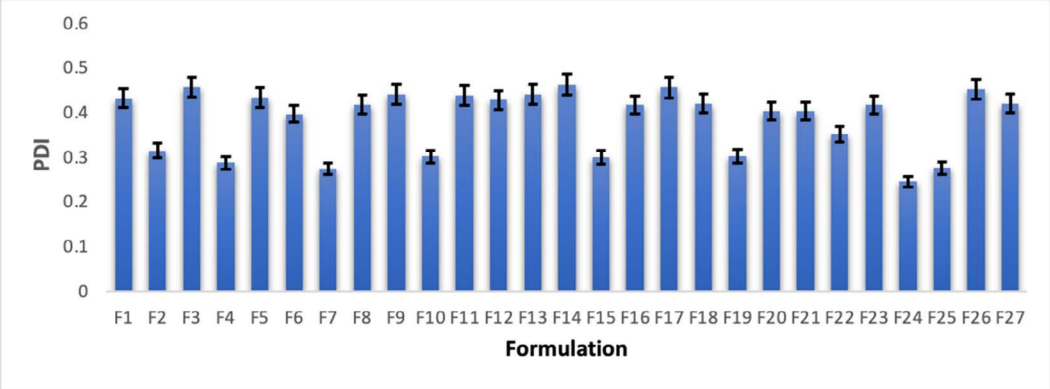

Figure 3: Polydispersity index for prepared ticagrelor nano micelles

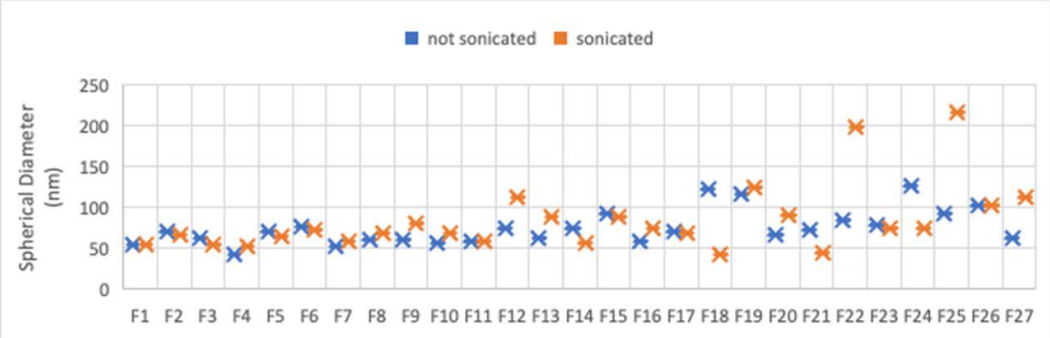

Figure 4: The effect of sonication on average spherical diameter (n=3) of ticagrelor nano micelles with sonication

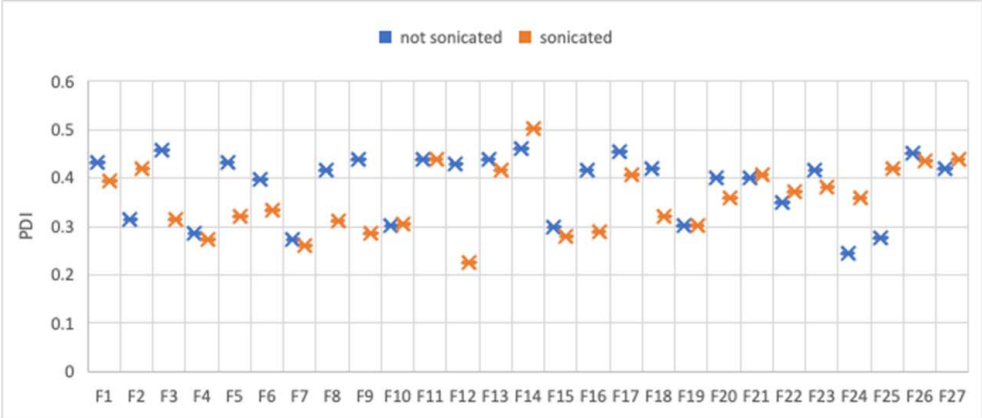

Figure 5: The effect of sonication on PDI of ticagrelor nano micelles with sonication

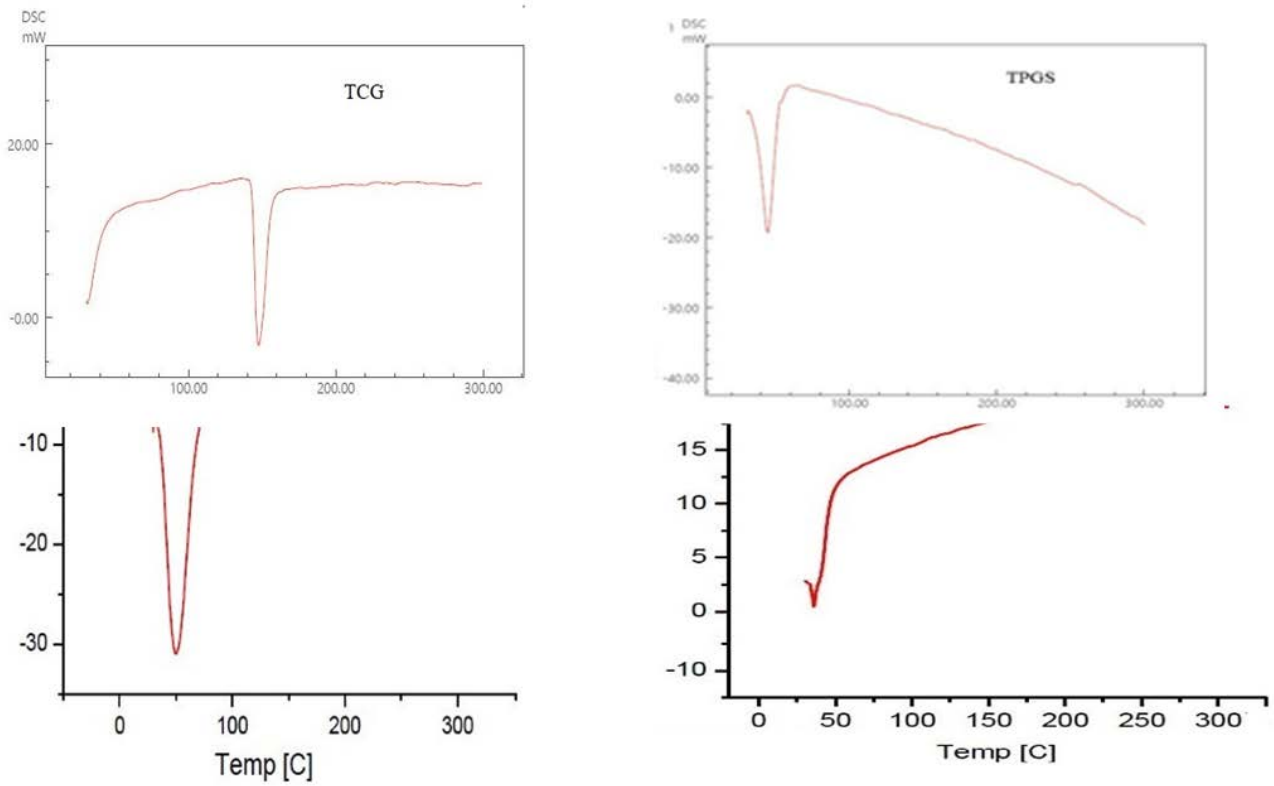

Figure 6: DSC image of (1) ticagrelor, (2) TPGS, (3) ticagrelor's physical mixture, (4) ticagrelor Nano micelle (F4)

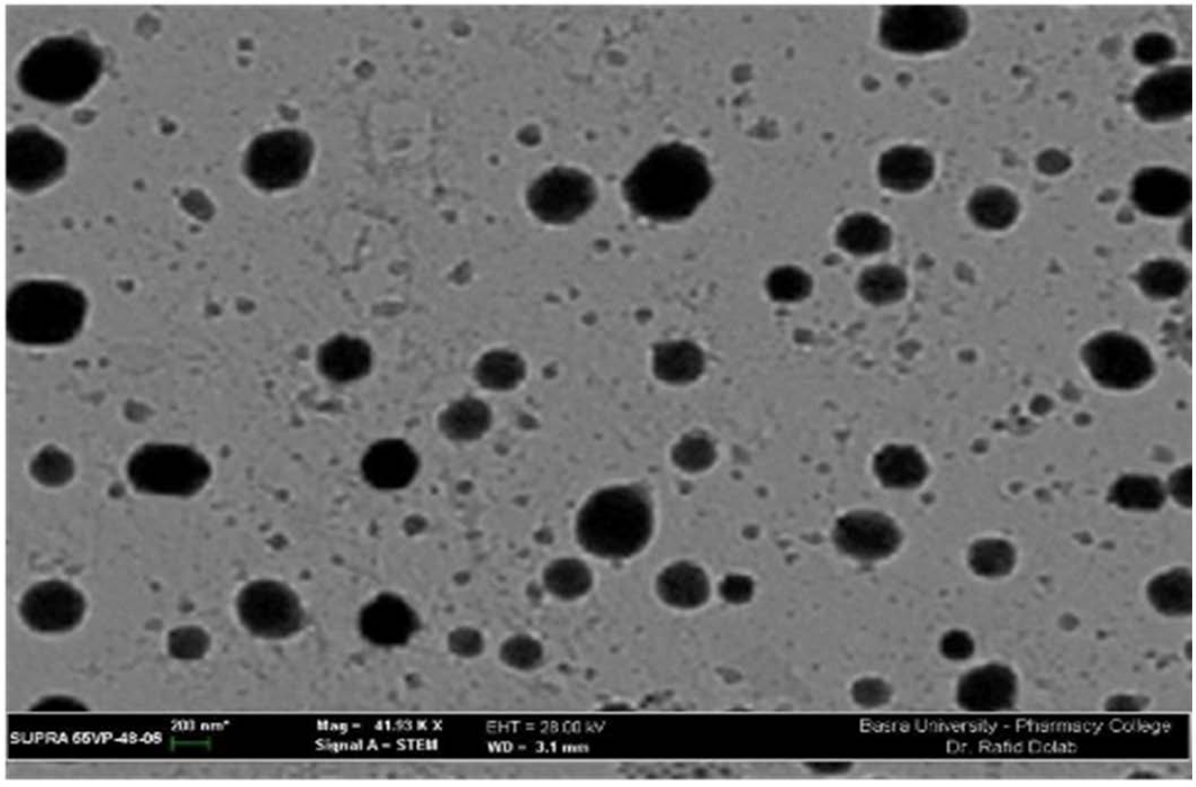

Figure 7:TEM image of selected F4 Ticagrelor Nano Micelles

Figure 7 illustrates the thermogram of ticagrelor nano micelles (F4). Only the TPGS melting point peak was visible; the TCG peak was not and indicated that the medication had been successfully dispersed and encapsulated in the TPGS core[29].

TEM Imaging

Figure 8 shows the TEM images of F4 nanomicelles, which revealed the spherical morphology[36,54] with no accumulation[45], representing the preferred shape for oral absorption.

Study on intestinal permeability under ex vivo conditions

Figure 9 represents the permeation of pure TCG and ticagrelor from nano micelles with a developed non-everted rat gut sac model. Figure 10 represents the intestinal motility recorded by the Iworx system. In this experiment, We chose rats because their physiological and environmental circumstances are similar to those of humans[69]. Male rats were chosen for this investigation because the female sex hormone significantly controls gastrointestinal motility, but the male sex hormone does not[70]. The intestinal smooth muscle contraction has been registered because gastrointestinal motility is one of the parameters influencing the absorption of medications taken orally[24]. The displacement transducer 475 serves as a sensor that transforms the contraction of this little section into an electrical or vibrational signal, which is then recorded in a particular chromatogram by a computer integrated into the I Worx system, as shown in figure 10[71]. The Tyrode solution contains the primary salts required for tissue culture. Its purpose is to maintain pH while supplying the intestine segment's cells with crucial ions and water to replicate the in vivo human environment and preserve the tissue's integrity during the experiment[72]. The jejunum was selected for this experiment for reasons, including its high concentration of the efflux transporter p-GP[73] and length related to the remained parts of the small intestine[74], so it will provide more remaining time and a higher drug-absorption surface area. Oral absorption necessitates the permeation of medicines through the intestinal mucosa. Lipid bilayer, pores, and drug's chemical structure are roleplaying. The top mucosal layer reduces the diffusion of drugs with high Lipophilicity or positive charges. The lipid and membrane pore also impact medicines' trans cellular and Para cellular penetration. In general, the membrane has amphiphilic molecular characteristics[75]. As outcomes, the pharmaceutical preparations should be lipophilic and hydrophilic to a certain extent for successful membrane permeation[76]. This property was present in ticagrelor Nano micelles result of TPGS. Intestinal motility is caused by depolarization of the membrane, which leads to the opening of a voltage-gated calcium channel and contraction of the intestinal smooth muscle[77]. This motion also influences diffusion because it aids in pushing luminal contents along with the GIT. Segmental motion keeps the greatest concentration of drug at the mucosal surface and then fluxes the drug from the mucosal surface to a lower concentration gradient[4]. The obtained finding revealed that ticagrelor nano micelles cause a significant ($p<0.05$) increase the medicine permeation to a more considerable extent than pure ticagrelor. When combined with amprenavir (a poorly water-soluble P.gp substrate) that impacts permeability in a concentration-dependent manner[78], this effect results from the following: TPGS had sufficient Lipophilicity and sufficient hydrophilicity for membrane permeation and delivered TCG to the blood, TPGS increase the water dissolution of drug and makes inhibition of p.gp efflux transporter[21]. The nanocarrier should cross the mucous layer, then partition in the membrane and Drug release from micelles happens. There are no Nano particulates in the tyrode solution, according to DLS examination, which indicates that the release of TCG from micelle has occurred.

The equation (2) and (3) show the obtained mathematic model of the kinetic study of the TCG diffusion for raw and TCG Nano micelles. As the slope for the raw is negative and approach to zero (-0.0053) that reveal to the rate is nearly zero order with slight decrease, this effect could be resulted from a decrease in the concentration of TCG inside intestinal segment with time. While the TCG nanomicelles showed a slope of (0.0745) that indicates there is increase in the rate of diffusion with time, this increase might be caused by the increase in the inhibition of the p-GP with time as more TPGS introduce with time.

$Y = -0.0053. X + 0.5866$ Equation …… (2)

$Y = 0.0745. X + 1.2325$ Equation……. (3)

The high affinity of TPGS to P-GP resulting in a liberation of ticagrelor from TPGS micelles. Due to hydrophobic bond between the drug molecule and the phospholipid in the biological membrane, it will experience high retention in the intestinal epithelium; this retention is necessitated for successful drug molecule diffusion along a concentration gradient from the apex to the basolateral side, causing oral absorption improvement[65]. The improvement in oral absorption by TPGS nano micelles was reported with drugs belonging to BCS class IV, such as curcumin[33], lopinavir[29], aripiprazole[44] and some anticancer drugs[54].

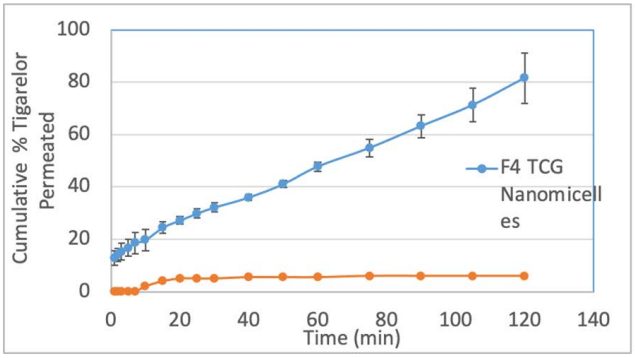

Figure 8: Ticagrelor permeation from nano micelles (F4) (blue line) compared with raw ticagrelor (Orange line)

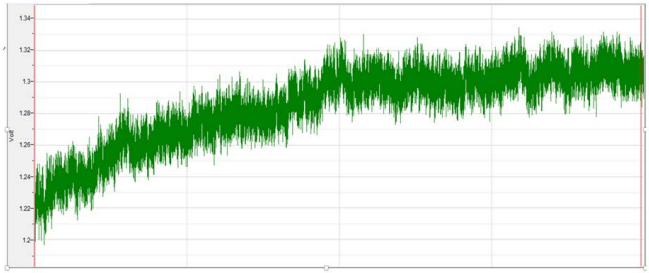

Figure 9: Intestinal tension measured by I Worx system during ex vivo permeation of TCG nanomicelles

Stability study

Storage stability

This test was conducted at ambient temperature to examine selected ticagrelor nano micelles' physical stability and storage behavior.

It was intended to demonstrate the kinetic stability of this micelles system[66]. There were no signs of sedimentation or liquid separation, and the nano micelle formulations remained clear for three months when observed by visualization. Also, as specified in table 4, there is no more significant rise in particle size and PDI after three months. The TPGS dispersion system was highly stable, with outstanding clarity and no evidence of precipitation[79,80]. The stability of ticagrelor nano micelles decreases as the temperature approaches 40 degrees Celsius. After 24 hours, a turbid formulation has formed because the high temperature negatively impacts the stability of TPGS micelles[52]. At high temperatures of more than 40° C, TPGS micelles are susceptible to distortion and disintegration, resulting in turbidity in this colloidal dispersion[51] because as the temperature increases, the CMC increases, then the thermodynamic and kinetic stability decrease[41].

Table 5: Effect of storage on particle size and PDI of selected F4 and F4 with sonication

Items	P. S	PDI
F4 at Zero time	42.03	0.287
F4 at the first month	45.07	0.294
F4 at the second month	47.04	0.314
F4 at the third month	50.05	0.358
F4 Sonicated at zero time	52.69	0.275
F4 Sonicated in the first month	55.69	0.293
F4 Sonicated in the second month	60.74	0.33
F4 sonicated at third months	65.13	0.4

pH stability:

This test was created to study the behavior of chosen ticagrelor nano micelles when passing through different pH media to simulate varying physiological pH in the GIT. The micelles should resist degradation and not release the loaded medicine when passing through different physiological pH to deliver the drug safely to the systemic circulation[41]. TPGS micelles are stable in an acidic stomach pH of 1.2[33,44] and stable in the intestinal pH, indicating that TPGS micelles are resistant to various pH changes and produce stable micelles capable of safely transporting medicines to the circulation[44,81,82] After two hours of incubation at 37c, particle size and PDI of ticagrelor nano micelles increased. Still, it remains within the standard limit for good oral absorption, as displayed in table 5. There is no sign of sedimentation after 8 hours when observed by visualization. TPGS nano micelles were stable when TPGS concentration about 10 mg/ml at PH 1.2 at 37 c.[50] in the selected ticagrelor nano micelles the concentration of TPGS is 12mg/ml. Therefore, it is stable in human gastric and intestinal conditions.

Table 6: Effect of pH on particle size (p.s) and PDI of selected F4 ticagrelor nano micelles (mean SD, n=3)

Name	PS	PDI
pH 1.2	93 ± 0.106	0.42 ± 0.003
pH 7	61 ± 0.193	0.25 ± 0.015

Dilution stability:

The purpose of the dilution test was to determine how the particle size and distribution of certain ticagrelor nano micelles changed as a result of dilution. This test was done to see how the stability of the ticagrelor nano micelles was affected by volume expansion[66]. The results in table 6 explain that no significant effect on the particle size and PDI of ticagrelor nano micelles were diluted to the degree at which TPGS concentrations reached 2mg/mL and 1mg/mL, respectively. It can give an idea about the resistance of TPGS nano micelles against dilution effects. The nano micelles are stable at concentrations above the critical micelles concentration (CMC), while disintegration and morphological changes in nano micelles occur at concentrations below the CMC[41].

Table 7: Effect of dilution on average (n=3) of PS and PDI of selected F4

Items	P. S	PDI
F4 (1:6)	64 ± 4.56	0.385 ± 0.026
F4 (1:12)	68 ± 4.18	0.27 ± 0.077

CONCLUSIONS

Ticagrelor nano micelles were successfully created utilizing the design of the experiment to improve penetration through the intestinal membrane. We determined that the hydration component has the highest impact on the formulation. From this study, we noticed that F4 had the smallest particle size, narrowest size distribution, highest encapsulation efficiency, and high stability at ambient temperature for three months and when exposed to physiological circumstances such as dilution and pH changes while travelling through the gastrointestinal system. Moreover, ex vivo permeability research demonstrated that medication diffusion across the intestinal barrier was successful when manufactured as ticagrelor nano micelles, which was 16 times more effective than raw ticagrelor. Based on our first results, we feel that ticagrelor formulation as nano micelles might be a potential strategy for enhancing ticagrelor intestinal absorption.

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Competing Interest: None

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REFERENCES

1. Aulton ME, Taylor K. Aulton's pharmaceutics: the design and manufacture of medicines. 4th ed. ed. 2013, Edinburgh: Churchill Livingstone. 2013.
2. Alqahtani MS. Advances in oral drug delivery. Front Pharmacol 2021;12:618411.
3. Jain KK. Drug delivery systems. 2008;251.
4. Franklin MR. Remingtons Essentials of Pharmaceutics. Drug absorption, distribution, metabolism and excretion. London UK: Pharmaceutical Press 2012.
5. York P. Design of dosage form, in Aulton ’s Pharmaceutics, M.E.A.a.K.M. Taylor, Editor. ELSEVIER 2013.
6. Reinholz J, Landfester K, Mailänder V. The challenges of oral drug delivery via nanocarriers. Drug Deliv 2018;25(1):1694-705.
7. Bose A. Nanomicelles: Types, properties and applications in drug delivery. IET Nanobiotechnol 2021;15(1):19-27.
8. Chinju M, Kripa S. A Comprehensive Review on Polymeric Micelles. Int J Pharm Sci Rev Res 2020;61(2):36-9.
9. Tawfik SM. Recent Advances in Nanomicelles Delivery Systems. Nanomaterials 2021;11(1):70.
10. Mitra AK, Cholkar K, Mandal A. Emerging nanotechnologies for diagnostics, drug delivery and medical devices. William Andrew 2017.
11. Akkaif MA. A review of the effects of ticagrelor on adenosine concentration and its clinical significance. Pharmacol Rep 2021;73(6):1551-64.

12. Misumida N. Ticagrelor versus clopidogrel in East Asian patients with acute coronary syndrome: systematic review and meta-analysis. Cardiovasc Revasc Med 2018;19(6):689-94.
13. Husted S, Van Giezen J. Ticagrelor: the first reversibly binding oral P2Y12 receptor antagonist. Cardiovasc Therap 2009;27(4):259-74.
14. Son GH. Systemic design and evaluation of ticagrelor-loaded nanostructured lipid carriers for enhancing bioavailability and antiplatelet activity. Pharmaceutics 2019;11(5):222.
15. Homayun B, Lin X, Choi HJ. Challenges and Recent Progress in Oral Drug Delivery Systems for Biopharmaceuticals. Pharmaceutics 2019;11(3):129.
16. Ghadi R, Dand N. BCS class IV drugs: Highly notorious candidates for formulation development. J Contr Release 2017;248:71-95.
17. Danielak D. Karaźniewicz-Łada M, Główka F. Ticagrelor in modern cardiology-an up-to-date review of most important aspects of ticagrelor pharmacotherapy. Exp Opin Pharmacother 2018;19(2):103-12.
18. Nguyen TTL, Duong VA, Maeng HJ. Pharmaceutical formulations with P-glycoprotein inhibitory effect as promising approaches for enhancing oral drug absorption and bioavailability. Pharmaceutics 2021;13(7):1103.
19. Na YG. Development and evaluation of TPGS/PVA-based nanosuspension for enhancing dissolution and oral bioavailability of ticagrelor. Int J Pharmaceutics 2020;581:119287.
20. Na YG. Strategic approach to developing a self-microemulsifying drug delivery system to enhance antiplatelet activity and bioavailability of ticagrelor. Int J Nanomed 2019;14:1193-212.
21. Guo Y. The applications of Vitamin E TPGS in drug delivery. Eur J Pharm Sci 2013;49(2):175-86.
22. Zhang Z, Tan S, Feng SS. Vitamin E TPGS as a molecular biomaterial for drug delivery. Biomaterials 2012;33(19):4889-906.
23. Kumbhar P. D-α-tocopheryl polyethylene glycol succinate: A review of multifarious applications in nanomedicines. Open Nano 2022;6:100036.
24. Aungst BJ. Intestinal permeation enhancers. J Pharm Sci 2000;89(4):429-42.
25. Muthu MS. Development of docetaxel-loaded vitamin E TPGS micelles: formulation optimization, effects on brain cancer cells and biodistribution in rats. Nanomedicine 2012;7(3):353-64.
26. Cholkar K. Optimization of dexamethasone mixed nanomicellar formulation. AAPS PharmSciTech 2014;15(6):1454-67.
27. Cholkar K. Nanomicellar Topical Aqueous Drop Formulation of Rapamycin for Back-of-the-Eye Delivery. AAPS PharmSciTech 2015;16(3):610-22.
28. Cholkar K, Gilger BC, Mitra AK. Topical, Aqueous, Clear Cyclosporine Formulation Design for Anterior and Posterior Ocular Delivery. Transl Vis Sci Technol 2015;4(3):1.
29. Mohammad FH, Noori LK, Younis NM.Assessment of Nutritional habits among Mosul University Students regarding breakfast. 2023 Jan. Vol. 48, (1):96-.
30. Ali HA, Abbas FF, Younis NM. Mothers' knowledge and attitudes towards breastfeeding in Thi-Qar City, Iraq. Rawal Medical Journal. 2023 May 27;48(2):514-.
31. Younis NM, Ahmed MM, Dhahir NM. Prevalence of Covoravirus among Healthcare Workers. Int J Med Toxicol Legal Med 2021;24(1-2):267-9.
32. Ali HA, Abbas FF, Younis NM. Mothers' knowledge and attitudes towards breastfeeding in Thi-Qar City, Iraq. Rawal Medical Journal. 2023 May 27;48(2):514-.
33. Younis NM, Ibrahim RM, Idrees NH.Prevalence of snake bite among children in Nineveh Governorate/Iraq: A retrospective study. International Journal of Medical Toxicology & Legal Medicine. 2022;25(3and4):169-172.
34. Younis NM, Mahmoud M, Ahmed A, et al. University Students' Attitude Towards E-Learning. Bahrain Med Bull 2021;43(2):460-2.
35. Muwfaq YN, Ahmed MM, Abdulsalam RR. Assessing Quality of Life in Palliative Care. Bahrain Med Bull 2021;43(3):594-6.
36. Taher AK, Younis NM. Assessment the Effect of a Trans theoretical Model in Improving Behaviors Health Care workers related Electronic Hookah in Mosul City /Iraq. Rawal Medical Journal: 2023 Jan. Vol. 48, (1):228-.
37. Younis NM. Efficacy of Health Beliefs Model-Based Intervention in Changing Substance Use Beliefs among Mosul University Students: A Randomized Controlled Trial. Revis Bionatura 2022;7(2):35.
38. Al-Ghurairi SA, Younis NM, Ahmed MM. Prevalence of weight gain among students of Mosul University, Iraq during quarantine 2020. Rawal Medical Journal. 2022 Jul;47(3).
39. Abbas AS, Younis NM. Efficacy of Pender's Health Promotion-based Model on Intervention for Enhancing University of Mosul Hypertensive Employees' Eating Behaviors: A randomized Controlled Trial. Revis Bionatura 2022;7(3):35.
40. Ahmed MM, Younis NM, Abdulsalam RR. Assessment of Changes in Sleep Habits in Elementary Students During COVID_19 Lockdown. In J Med Tox Leg Med 2022;25(1-2):107-11.
41. Mukhlif HH, Younis NM. Evaluation of the association between internet addiction and fatigue among undergraduate students at universities in Mosul city, Iraq: A cross-sectional study. Rawal Med J 2022;47(4):829-33.
42. Adea MK, Lefta RM, Younis NM. Impact of psychosocial aspect parameters on psoriasis patients' quality of life at outpatient clinic in Al-Dewania City, Iraq. Rawal Medical Journal. 2022 Dec 11;47(4):892-.
43. Ibrahim RM, Idrees NH, Younis NM. Epidemiology of leukemia among children in Nineveh Province, Iraq. Rawal Medical Journal: 2023 Jan. Vol. 48, (1):137-.
44. Ali HA, Al-Waly LAM, Mukhlif HH, et al. Types of Congenital Anomalies among Children at Bint Al-Huda Teaching Hospital in Al-Nasiriyah City, South of Iraq. Bahrain Med Bull 2022;44(1):792-4.
45. Mahajan HS, Patil PH. Central composite design-based optimization of lopinavir vitamin E-TPGS micelle: In vitro characterization and in vivo pharmacokinetic study. Colloids Surf B Biointerfaces 2020;194:111149.
46. Sun C. Development of TPGS/F127/F68 mixed polymeric micelles: enhanced oral bioavailability and hepatoprotection of syringic acid against carbon tetrachloride-induced hepatotoxicity. Food Chem Toxicol 2020;137:111126.
47. Asadi A. Effect of sonication characteristics on stability, thermophysical properties, and heat transfer of nanofluids: A comprehensive review. Ultrasonics Sonochem 2019;58:104701.
48. Hasanzadeh H. Dual-frequency ultrasound activation of nanomicellar doxorubicin in targeted tumor chemotherapy. J Med Ultrasonics 2014;41(2):139-50.
49. Ji S. Curcumin-loaded mixed micelles: Preparation, characterization, and in vitro antitumor activity. J Nanotechnol 2018;2018:1-9.
50. Niu XQ. Mechanism investigation of ethosomes transdermal permeation. Int J Pharm 2019;1:100027.
51. AL Radhi MS, AL-Lami MS, Abdulsahib E. Preparation and characterization of diazepam loaded nanomicelles for pediatric intravenous dose adjustment. Int J Pharm Res 2021;13(1).
52. Keshari P, Sonar Y, Mahajan H. Curcumin loaded TPGS micelles for nose to brain drug delivery: in vitro and in vivo studies. Materials Technol 2019;34(7):423-32.

53. Jin IS. Physicochemical, pharmacokinetic, and toxicity evaluation of soluplus® polymeric micelles encapsulating fenbendazole. Pharmaceutics 2020;12(10):1000.
54. Doulab RA, Al-lami MS. A new Ex Vivo apparatus for assessing drug intestinal permeability by data acquisition assessment, U.S.P.a.T. Office, Editor 2022.
55. Al-lami RADMS. A new Ex Vivo Apparatus for Assessing Drug Intestine Permeability by DataAcquisition Assessment, in OHIOPUBLISHING & ACADEMICSERVICES US, Editor 2022.
56. Butt AI. Comparison of prokinetic activity of ranitidine and neostigmine alone and in combination-an in vitro study. PAFMJ 2021;71(Suppl-1):S66-70.
57. Ghezzi M. Polymeric micelles in drug delivery: An insight of the techniques for their characterization and assessment in biorelevant conditions. J Contr Rel 2021;332:312-36.
58. Derakhshandeh K, Fathi S. Role of chitosan nanoparticles in the oral absorption of Gemcitabine. Int J Pharm 2012;437(1-2):172-7.
59. Maheshwari M. Comparative bioavailability of curcumin, turmeric and Biocurcumax™ in traditional vehicles using non-everted rat intestinal sac model. J Funct Foods 2010;2(1):60-5.
60. Piazzini V. Enhanced dissolution, permeation and oral bioavailability of aripiprazole mixed micelles: In vitro and in vivo evaluation. Int J Pharm 2020;583:119361.
61. Alhakamy NA. Development and in vitro evaluation of 2-methoxyestradiol loaded polymeric micelles for enhancing anticancer activities in prostate cancer. Polymers 2021;13(6):884.
62. Yokoyama M. Incorporation of water-insoluble anticancer drug into polymeric micelles and control of their particle size. J Contr Rel 1998;55(2-3):219-29.
63. Lennernäs H. Regional intestinal drug permeation: Biopharmaceutics and drug development. Eur J Pharm Sci 2014;57:333-41.
64. Rogošić M, Mencer H, Gomzi Z. Polydispersity index and molecular weight distributions of polymers. Eur Polymer J 1996;32(11):1337-44.
65. Honary S, Zahir F. Effect of zeta potential on the properties of nano-drug delivery systems-a review (Part 2). Trop J Pharm Res 2013;12(2):265-73.
66. Kim MS. Enhanced solubility and oral absorption of sirolimus using D-α-tocopheryl polyethylene glycol succinate micelles. Artificial Cells Nanomed Biotechnol 2013;41(2):85-91.
67. Zuccari G. Increased water-solubility and maintained antioxidant power of resveratrol by its encapsulation in vitamin E TPGS micelles: A potential nutritional supplement for chronic liver disease. Pharmaceutics 2021;13(8):1128.
68. Zuccari G. D-α-Tocopherol-Based Micelles for Successful Encapsulation of Retinoic Acid. Pharmaceuticals 2021;14(3):212.
69. Shahid N. Fabrication of thiolated chitosan based biodegradable nanoparticles of ticagrelor and their pharmacokinetics. Polymers Polymer Comp 2022;30:09673911221108742.
70. Rathod S, Bahadur P, Tiwari S. Nanocarriers based on vitamin E-TPGS: Design principle and molecular insights into improving the efficacy of anticancer drugs. Int J Pharm 2021;592:120045.
71. Al-Saffar A. Utility of animal gastrointestinal motility and transit models in functional gastrointestinal disorders. Best Pract Res Clin Gastroenterol 2019;40:101633.
72. Chen TS. Effects of sex steroid hormones on gastric emptying and gastrointestinal transit in rats. Am J Physiol Gastrointestinal Liver Physiol 1995;268(1):G171-6.
73. Afzal A. Effect of paroxetine on intestinal motility in the presence of ondansetron. Pak J Pharm Sci 2020;33(3):1169-72.
74. Gilani AH. Pharmacological basis for the use of turmeric in gastrointestinal and respiratory disorders. Life Sci 2005;76(26):3089-105.
75. Suzuki H, Sugiyama Y. Role of metabolic enzymes and efflux transporters in the absorption of drugs from the small intestine. Eur J Pharm Sci 2000;12(1):3-12.
76. Kiela PR, Ghishan FK. Physiology of Intestinal Absorption and Secretion. Best Pract Res Clin Gastroenterol 2016;30(2):145-59.
77. Schoultz I, Keita AV. The intestinal barrier and current techniques for the assessment of gut permeability. Cells 2020;9(8):1909.
78. Dahlgren D, Lennernäs H. Intestinal permeability and drug absorption: Predictive experimental, computational and in vivo approaches. Pharmaceutics 2019;11(8):411.
79. Evans ED, Mangel AW. Depolarization-Stimulated Contractility of Gastrointestinal Smooth Muscle in Calcium-Free Solution: A Review. ISRN Gastroenterol 2011;2011:692528.
80. Brouwers J. Intraluminal drug and formulation behavior and integration in in vitro permeability estimation: a case study with amprenavir. J Pharm Sci 2006;95(2):372-83.
81. Lee Y, Choi SQ. Quantitative analysis for lipophilic drug transport through a model lipid membrane with membrane retention. Eur J Pharm Sci 2019;134:176-84.
82. Owen SC, Chan DP, Shoichet MS. Polymeric micelle stability. Nano Today 2012;7(1):53-65.
83. Aggarwal N, Goindi S, Mehta SD. Preparation and evaluation of dermal delivery system of griseofulvin containing vitamin E-TPGS as penetration enhancer. Aaps Pharmscitech 2012;13(1):67-74.
84. Sipos B. Development of dexamethasone-loaded mixed polymeric micelles for nasal delivery. Eur J Pharm Sci 2021;166:105960.
85. Sant VP, Smith D, Leroux JC. Enhancement of oral bioavailability of poorly water-soluble drugs by poly (ethylene glycol)-block-poly (alkyl acrylate-co-methacrylic acid) self-assemblies. J Contr Release 2005;104(2):289-300.

GRAPHICAL ABSTRACT