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ORIGINAL ARTICLE

Synthesis, Characterization, and Antimicrobial Evaluation of Some Novel Hydrazinecarbothioamides

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ABSTRACT

Objectives: This study focused on synthesizing and characterizing novel thiosemicarbazide derivatives containing a 1,2,4-triazole moiety and evaluating their antimicrobial activity against several bacterial strains. The research aimed to identify key structural features that enhance antimicrobial efficacy through structure-activity relationship analysis and identify the minimum inhibitory concentration (MIC) of the most potent compounds to assess their potential for further development as antimicrobial agents.

Materials and Methods: Nine novel thiosemicarbazide derivatives containing a 1,2,4-triazole moiety were synthesized by reacting 1,2,4-triazole derivatives with thiosemicarbazide precursors, and the products were characterized using infrared spectroscopy, proton nuclear magnetic resonance (^{1}H-NMR), carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectroscopy, and elemental analysis. The antimicrobial activity of these compounds (**5a-i**) was tested against *Klebsiella pneumoniae (K. pneumoniae), Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa (P. aeruginosa),* using microdilution, disk diffusion, and broth microdilution methods. Dimethyl sulfoxide was used as a negative control, and Vancomycin and Meropenem were used as positive controls, with all results converted to µM for consistent analysis.

Results: The synthesized thiosemicarbazide derivatives (**5a-i**) were confirmed to be structurally correct through Fourier-transform infrared spectroscopy, ^{1}H-NMR, and ^{13}C-NMR spectroscopy. Among the tested compounds, **5e** (4-bromophenyl) and **5g** (n-propyl) showed significant antimicrobial activity, with **5g** exhibiting the strongest effects against *S. aureus* and *P. aeruginosa*. Other derivatives, such as **5b** (4-NO_2Ph), **5c** (4-FPh), and **5d** (4-ClPh), showed moderate activity, while no significant activity was observed against *K. pneumoniae* or *E. faecalis*.

Conclusion: The study successfully synthesized a series of novel thiosemicarbazide derivatives with a 1,2,4-triazole moiety and evaluated their antimicrobial potential. Compounds **5e** and **5g** exhibited significant antibacterial activity, particularly against *S. aureus* and *P. aeruginosa*, with MIC values in the low micromolar range. These findings suggest that the compounds hold promise as potential antimicrobial agents, and further studies should focus on optimizing their efficacy and exploring their mechanism of action.

Keywords: Synthesis, hydrazinecarbothioamide, 1,2,4-triazole, antimicrobial activity

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INTRODUCTION

The escalating prevalence of antibiotic-resistant bacteria, fueled by the misuse of antibacterial agents, has become a critical global health crisis.[1,2] As these resistant strains continue to evolve, an increasing number of infections are no longer responding to conventional treatments. Alarmingly, even last-resort antibiotics are losing their efficacy in some cases.[3-5] This surge in antimicrobial resistance underscores the urgent need for new therapeutic agents, making the development of novel treatments a top priority in medicinal chemistry today.[6-8]

Thiosemicarbazides are vital functional groups in medicinal chemistry, known for their role in forming biologically active heterocyclic rings such as 1,3,4-thiadiazoles, 4-thiazolidinones, and 1,2,4-triazole-3-thiones.[9-11] Numerous studies have highlighted the diverse biological activities exhibited by thiosemicarbazides. Triazoles, defined by a five-membered ring containing three nitrogen atoms and two carbon atoms, are particularly noteworthy in this context. Triazole derivatives have garnered significant attention due to their broad spectrum of biological activities.[9,12-16]

Several widely used pharmaceuticals incorporate the 1,2,4-triazole scaffold, including the antifungal agents fluconazole, itraconazole, and voriconazole, the antimigraine drug rizatriptan, and the antiviral drug ribavirin (Figure 1). Additionally, the inclusion of fluorine atoms in drug design has become increasingly popular.[17-20] Fluorine's unique properties, such as its ability to impart diverse physicochemical attributes and its minimal steric impact, make it an attractive choice for enhancing drug interactions with biological systems. Its high electronegativity also significantly alters the physical and chemical properties of molecules, making fluorine incorporation a powerful strategy in medicinal chemistry.[21,22]

Motivated by the urgent need to overcome antibacterial resistance, our primary aim was to design agents based on thiosemicarbazide scaffolds bearing a 1,2,4-triazole heterocycle, selected for their well-established medicinal relevance outlined above.

This study reports the design, synthesis, structural characterization, and antimicrobial evaluation of nine novel thiosemicarbazides (**5a-i**), each incorporating a 1,2,4-triazole moiety (Figure 2). The antimicrobial efficacy of the synthesized compounds was tested against a panel of bacterial strains, including *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (BAA-2146), and *Escherichia coli* (ATCC 25922).

MATERIALS AND METHODS

Materials

Melting points were recorded on a STUART SMP40. Infrared (IR) spectra were recorded on a Shimadzu Fourier-transform infrared (FT-IR) spectrometer using KBr pellets. Affinity-1 FT-IR spectroscopy instrument and an Alpha Bruker FT-IR spectrometer. Proton nuclear magnetic resonance (^{1}H-NMR) and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra were measured on a Bruker spectrometer in dimethyl sulfoxide (DMSO)-d_6 solution at 500 Megahertz (MHz) and 125 MHz, respectively. Chemical shifts (δ) were reported in ppm, and coupling constants (J) were recorded in hertz (Hz).

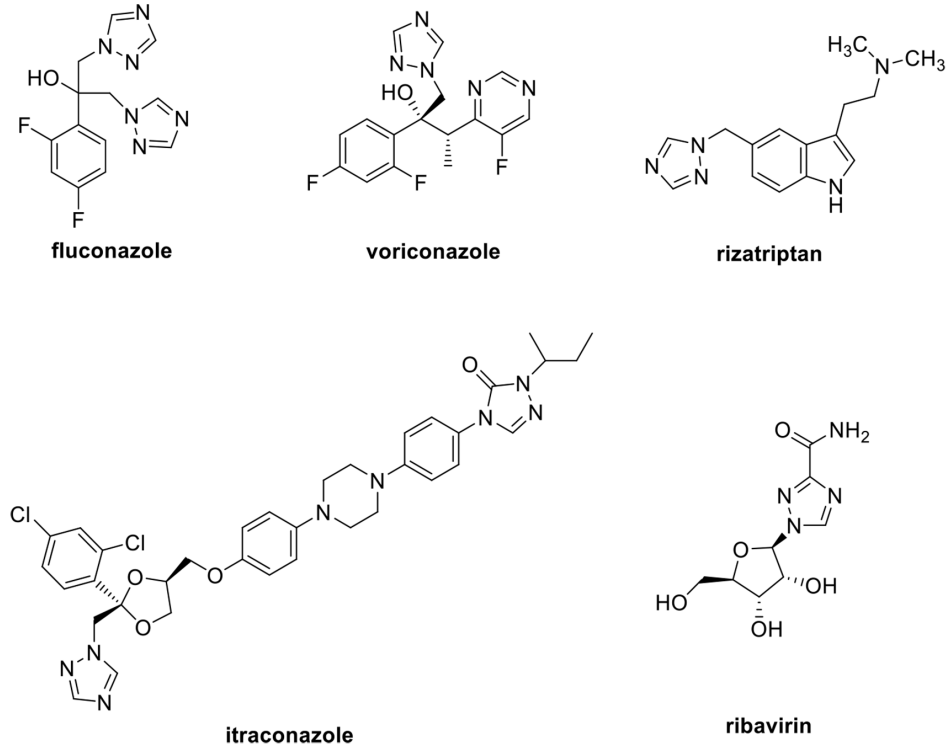


Figure 1. Chemical structures of some drugs containing the 1,2,4-triazole structure

Elemental analysis was recorded using Leco CHNS-932. The reactions were monitored using thin-layer chromatography on aluminum plates, which have a silica gel Kieselgel 60 F 254 layer of thickness 0.25 mm (Merck), using ultraviolet light as a visualizing agent. All reagents and solvents were purchased from Merck, Fluka, and Sigma-Aldrich and were used without further purification.

Chemical synthesis

Synthesis of N-(4-fluorophenyl)-2-(furan-2-carbonyl) hydrazine-1-carbothioamide (1)

N-(4-Fluorophenyl)-2-(furan-2-carbonyl)hydrazine-1-carbothioamide (1) was synthesized with a good yield of 86% through the reaction of equimolar amounts (0.005 mol) of 2-furoic acid hydrazide and 4-fluorophenylisothiocyanate. The reaction proceeded smoothly under reflux in boiling ethanol.[23]

Synthesis of 4-(4-fluorophenyl)-5-(furan-2-yl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (2)

A solution of compound 1 (0.005 mol) was prepared in 2N NaOH, and the solution was refluxed for 4-5 hours. Afterward, the solution was neutralized using 12.5% hydrochloric acid (HCl). Lastly, the product was filtered and washed with distilled water (Yield: 93%).[23]

Synthesis of ethyl ((4-fluorophenyl)-5-(furan-2-yl)-4H-1,2,4-triazole-3-yl)sulfanyl)acetate (3)

A mixture of 0.005 mol of compound 2, K_2CO_3, and 0.0055 mol of ethylbromoacetate ($BrCH_2COOEt$), in ethanol, was refluxed for 5 hours. After completion, the reaction mixture was cooled and poured onto ice water, yielding ethyl ((4-*fluorophenyl)-5-(furan-2-yl)-4H-1,2,4-triazole-3-yl)sulfanyl)acetate* (3) with a 75% yield.[24]

Synthesis of 2-[[4-(4-fluorophenyl)-5-(furan-2-yl)-4H-1,2,4-triazol-3-yl]sulfanyl]acetohydrazide (4)

A mixture of compound 3 (0.005 mol), ethanol, and $NH_2NH_2.H_2O$ (0.025 mol) was heated under reflux in ethanol for 5-6 hours to yield 2-[[4-(4-fluorophenyl)-5-(furan-2-yl)-4*H*-1,2,4-triazol-3-yl]sulfanyl] acetohydrazide (4), with a 61.1% yield.

The analytical and spectral data were reported in our previous study.[25]

Synthesis of N-(substituted)-2-{[(4-(4-fluorophenyl)-5-(furan-2-yl)-4H-1,2,4-triazol-3-yl)sulfanyl]acetyl}hydrazine-1-carbothioamides ***(5a-i)***

0.005 mol of compound 4 is dissolved in ethanol, and 0.005 mol of appropriate isothiocyanate is added to it. It is boiled in a water bath under reflux for 4 hours. At the end of the time, the precipitated product **(5a-i)** is filtered, dried, and the yield is calculated.

N-(Phenyl)-2-{[(4-(4-fluorophenyl)-5-(furan-2-yl)-4H-1,2,4-triazol-3-yl)sulfanyl]acetyl}hydrazine-1-carbothioamide (5a)

White powder, mp 178-179 °C, yield: 71.42%, Anal. Calcd. for: $C_{21}H_{17}FN_6O_2S_2$, C, 53.83; H, 3.66; N, 17.94%; Found: C, 53.46; H, 3.785; N, 17.88%. FT-IR υmax (cm^{-1}): 3300, 3252 (N-H), 3146, 3096, 3046 (ar. C-H), 2930, 2834 (al. C-H), 1706 (C=O). ^{1}H-NMR (500 MHz) (DMSO-d_6) δ (ppm): 10.40 (s, 1H, CONH), 9.78 (s, 1H, NH), 9.75 (s, 1H, NH), 7.78 (d, *J*: 1.64 Hz, 1H, furan C_5-H), 7.67-7.61 (m, 2H, 4FPh$C_{2,6}$-H), 7.55 (d, *J*: 7.73 Hz, 2H, Ph $C_{2,6}$-H), 7.49 (t, *J*: 8.77 Hz, 2H, 4FPh$C_{3,5}$-H), 7.35 (t, *J*: 7.73 Hz, 2H, Ph $C_{3,5}$-H), 7.18 (t, *J*: 7.73 Hz, 1H, Ph C_4-H), 6.54 (dd, *J*: 3.45; 1.64 Hz, 1H, furan C_4-H), 6.24 (d, *J*: 3.45 Hz, 1H, furan C_3-H), 3.99 (s, 2H, S$\underline{C}H_2$). ^{13}C-NMR (APT) (125 MHz) (DMSO-d_6) δ (ppm): 181.2 (C=S), 167.2 (C=O), 163.2 (d, *J*: 148 Hz; 4FPh C_4), 151.7 (triazole C_2), 147.9 (triazole C_5), 145.4 (furan C_5), 141.2 (furan C_2), 139.4, 130.7 (d, *J*: 9.25 Hz, 4FPh C_2), 130.0 (d, *J*: 2.69 Hz, 4FPh C_1), 128.4, 126.2, 125.6, 117.5 (d, *J*: 23.36 Hz; 4FPh C_3), 112.2 (furan C_3), 112.0 (furan C_4), 35.2 (-S$\underline{C}H_2$).

N-(4-Nitrophenyl)-2-{[(4-(4-fluorophenyl)-5-(furan-2-yl)-4H-1,2,4-triazol-3-yl)sulfanyl]acetyl}hydrazine-1-carbothioamide (5b)

Light yellow powder, mp 182 °C, yield: 64.93%, Anal. Calcd. for: $C_{21}H_{16}FN_7O_4S_2$ C, 49.12; H, 3.14; N, 19.09%; Found: C, 49.21; H, 3.259; N, 19.04%. FT-IR υmax (cm^{-1}): 3280, 3210 (N-H), 3147, 3101 (ar. C-H), 2928, 2837 (al. C-H), 1721 (C=O), ^{1}H-NMR (500 MHz) (DMSO-d_6) δ (ppm): 10.52 (s, 1H, CONH), 10.22 (s, 1H, NH), 10.09 (s, 1H, NH), 8.23 (d, *J*: 8.73 Hz, 2H, 4-NO_2Ph $C_{2,6}$-H), 8.03

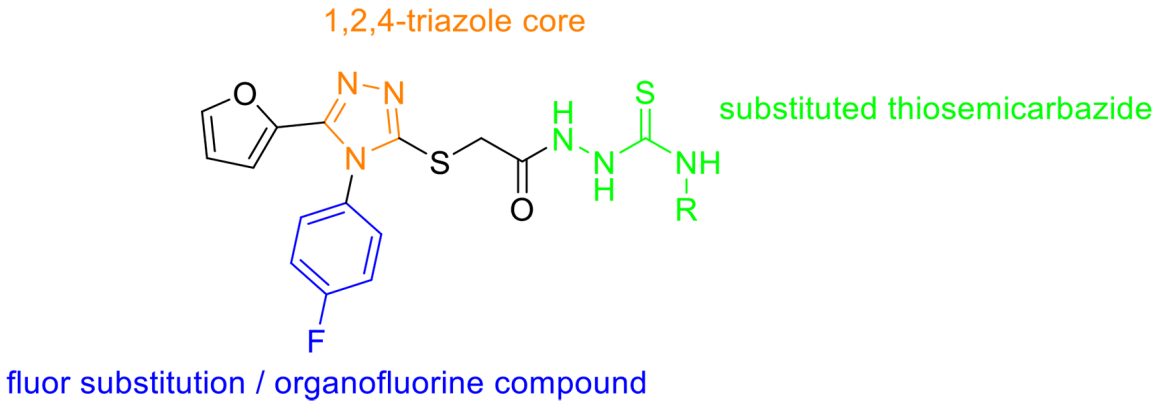


Figure 2. Structure of synthesized compounds

(broad s, 2H, 4-NO_2Ph $C_{3,5}$-H), 7.79 (s, 1H, furan C_5-H), 7.68-7.62 (m, 2H, 4FPh$C_{2,6}$-H), 7.49 (t, *J*: 8.54 Hz, 2H, 4FPh$C_{3,5}$-H), 6.54 (m, 1H, furan C_4-H), 6.26 (d, *J*: 3.56 Hz, 1H, furan C_3-H); 3.98 (s, 2H, S$C\underline{H}_2$). ^{13}C-NMR (APT) (125 MHz) (DMSO-d_6) δ (ppm): 180.9 (C=S), 167.5 (C=O), 163.2 (d, *J*: 248.64 Hz, 4FPh C_4), 151.7 (triazole C_2), 147.9 (triazole C_5), 145.8 (4-NO_2Ph C_4), 145.5 (furan C_5), 144.0 (4-NO_2Ph C_1), 141.2 (furan C_2), 130.7 (d, *J*: 9.26 Hz, 4FPh C_2), 130.3 (d, *J*: 2.75 Hz, 4FPh C_1), 125.2 (4-NO_2Ph C_3), 124.1 (4-NO_2Ph C_2), 117.5 (d, *J*: 23.31 Hz, 4FPh C_3), 112.2 (furan C_3), 112.1 (furan C_4), 35.2 (-S$\underline{C}H_2$).

N-(4-Fluorophenyl)-2-{[(4-(4-fluorophenyl)-5-(furan-2-yl)-4H-1,2,4-triazol-3-yl)sulfanyl]acetyl}hydrazine-1-carbothioamide (5c)

White powder, mp 178 °C, yield: 77.24%, Anal. Calcd. for: $C_{21}H_{16}F_2N_6O_2S_2$, C, 51.84; H, 3.31; N, 17.27%; Found: C, 51.48; H, 3.444; N, 17.17%. FT-IR υmax (cm^{-1}): 3298 (N-H), 3122, 3095 (ar. C-H), 2929, 2833 (al. C-H), 1715 (C=O). ^{1}H-NMR (500 MHz) (DMSO-d_6) δ (ppm): 10.33 (s, 1H, CONH), 9.74 (s, 1H, NH), 9.69 (s, 1H, NH), 7.69 (d, *J*: 1.82 Hz, 1H, furan C_5-H), 7.58-7.52 (m, 2H, 4FPh$C_{2,6}$-H), 7.46-7.37 (m, 4H, 4FPh$C_{2,3,5,6}$-H), 7.09 (t, *J*: 8.75 Hz, 2H, 4FPh$C_{3,5}$-H), 6.45 (dd, *J*: 3.46, 1.82 Hz, 1H, furan C_4-H), 6.15 (d, *J*: 3.46 Hz, 1H, furan C_3-H), 3.89 (s, 2H, S$C\underline{H}_2$).^{13}C-NMR (APT) (125 MHz) (DMSO-d_6) δ (ppm): 181.5 (C=S), 167.2 (C=O), 163.2 (d, *J*: 248 Hz, 4FPh C_4), 160.0 (d, *J*: 248 Hz, 4FPh C_4), 151.8 (triazole C_2), 147.9 (triazole C_5), 145.4 (furan C_5), 141.2 (furan C_2), 135.7 (d, *J*: 2.77 Hz, 4FPh C_1), 130.6 (d, *J*: 9.27 Hz, 4FPh C_2), 130.0 (d, *J*: 2.77 Hz, 4FPh C_1), 117.5 (d, *J*: 23.05 Hz, 4FPh C_3), 115.1 (d, *J*: 23.05 Hz, 4FPh C_3), 112.2 (furan C_3), 112.0 (furan C_4), 35.2 (-S$\underline{C}H_2$).

N-(4-Chlorophenyl)-2-{[(4-(4-fluorophenyl)-5-(furan-2-yl)-4H-1,2,4-triazol-3-yl)sulfanyl]acetyl}hydrazine-1-carbothioamide (5d)

White powder, mp 214 °C, yield: 82.66%, Anal. Calcd. for: $C_{21}H_{16}ClFN_6O_2S_2$, C, 50.15; H, 3.21; N, 16.71%; Found: C, 50.04; H, 3.268; N, 16.75%. FT-IR υmax (cm^{-1}): 3295 (N-H), 3153, 3097 (ar. C-H), 2957 (al. C-H), 1709 (C=O). ^{1}H-NMR (500 MHz) (DMSO-d_6) δ (ppm): 10.42 (s, 1H, CONH), 9.89 (s, 1H, NH), 9.81 (s, 1H, NH), 7.78 (d, *J*: 1.24 Hz, 1H, furan C_5-H), 7.68-7.60 (m, 2H, 4FPh$C_{2,6}$-H), 7.59 (d, *J*: 8.7 Hz, 2H, 4-ClPh $C_{2,6}$-H), 7.49 (t, *J*: 8.60 Hz, 2H, 4FPh$C_{3,5}$-H), 7.40 (d, *J*: 8.7 Hz, 2H, 4-ClPh $C_{3,5}$-H), 6.54 (dd, *J*: 3.56; 1.78 Hz, 1H, furan C_4-H), 6.25 (d, *J*: 3.56 Hz, furan C_3-H), 3.97 (s, 2H, S$C\underline{H}_2$). ^{13}C-NMR (APT) (125 MHz) (DMSO-d_6) δ (ppm): 181.2 (C=S), 167.3 (C=O), 163.2 (d, *J*: 248 Hz, 4FPh C_4), 151.8 (triazole C_2), 147.9 (triazole C_5), 145.5 (furan C_5), 141.2 (furan C_2), 138.4 (4-ClPh C_1), 130.7 (d, *J*: 9.27 Hz, 4FPh C_2), 130.0 (d, *J*: 2.77 Hz, 4FPh C_1), 129.6 (4-ClPh C_4), 128.3 (4-ClPh $C_{3,5}$), 127.8 (4-ClPh $C_{3,5}$), 117.5 (d, *J*: 23.05 Hz, 4FPh C_3), 112.2 (furan C_3), 112.0 (furan C_4), 35.2 (-S$\underline{C}H_2$).

N-(4-Bromophenyl)-2-{[(4-(4-fluorophenyl)-5-(furan-2-yl)-4H-1,2,4-triazol-3-yl)sulfanyl]acetyl}hydrazine-1-carbothioamide (5e)

White powder, mp 210 °C, yield: 61.66%, Anal. Calcd. for: $C_{21}H_{16}BrFN_6O_2S_2.2H_2O$ C, 43.23; H, 3.46; N, 14.40%; Found: C, 43.71; H, 3.017; N, 14.77%. FT-IR υmax (cm^{-1}): 3293 (N-H), 3157, 3098 (ar. C-H), 2943 (al. C-H), 1708 (C=O). ^{1}H-NMR (500 MHz) (DMSO-d_6) δ (ppm): 10.34 (s, 1H, CONH), 9.82 (s, 1H, NH), 9.71 (s, 1H, NH), 7.70 (d, *J*: 1.79 Hz, 1H, furan C_5-H), 7.58-7.53 (m, 2H, 4FPh$C_{2,6}$-H), 7.47-7.41 (m, 6H, 4FPh$C_{2,6}$-H and 4BrPh$C_{2,3,5,6}$-H), 6.46 (dd, *J*: 3.5; 1.79 Hz, 1H, furan C_4-H), 6.17 (d, *J*: 3.5 Hz, 1H, furan C_3-H), 3.89 (s, 2H, S$C\underline{H}_2$).^{13}C-NMR (APT) (125 MHz) (DMSO-d_6) δ (ppm): 181.2 (C=S), 167.3 (C=O), 163.2 (d, *J*: 248.64 Hz, 4FPh C_4), 151.8 (triazole C_2), 147.9 (triazole C_5), 145.5 (furan C_5), 141.2 (furan C_2), 138.8, 131.3, 130.6 (d, *J*: 9.26 Hz, 4FPh C_2), 130.0 (d, *J*: 2.75 Hz, 4FPh C_1), 117.5 (d, *J*: 23.31 Hz, 4FPh C_3), 112.2 (furan C_3), 112.0 (furan C_4), 35.2 (-S$\underline{C}H_2$).

N-(Ethyl)-2-{[(4-(4-fluorophenyl)-5-(furan-2-yl)-4H-1,2,4-triazol-3-yl)sulfanyl]acetyl}hydrazine-1-carbothioamide (5f)

White powder, mp 180 °C, yield: 79.36%, Anal. Calcd. for: $C_{17}H_{17}FN_6O_2S_2$, C, 48.56; H, 4.08; N, 19.99%; Found: C, 48.34; H, 4.218; N, 19.93%. FT-IR υmax (cm^{-1}): 3311 (N-H), 3050 (ar. C-H), 2972, 2934, 2869 (al. C-H), 1709 (C=O). ^{1}H-NMR (500 MHz) (DMSO-d_6) δ (ppm): 10.21 (s, 1H, CONH), 9.33 (s, 1H, NH), 8.24 (s, 1H, NH), 7.79 (d, *J*: 1.8 Hz, 1H, furan C_5-H), 7.67-7.57 (m, 2H, 4FPh$C_{2,6}$-H); 7.49 (t, *J*: 8.8 Hz, 2H, 4FPh$C_{3,5}$-H), 6.55 (dd, *J*: 3.5; 1.8 Hz, 1H, furan C_4-H), 6.24 (d, *J*: 3.5 Hz, 1H, furan C_3-H), 3.90 (s, 2H, S$C\underline{H}_2$), 3.56 (p, *J*: 7 Hz, 2H, -$C\underline{H}_2$-CH_3), 1.12 (t, *J*: 7 Hz, 3H, -CH_2-$C\underline{H}_3$). ^{13}C-NMR (APT) (125 MHz) (DMSO-d_6) δ (ppm): 181.5 (C=S), 167.0 (C=O), 163.3 (d, *J*: 248.5 Hz, 4FPh C_4), 152.1 (triazole C_2), 147.9 (triazole C_5), 145.5 (furan C_5), 141.1 (furan C_2), 130.6 (d, *J*: 9.16 Hz, 4FPh C_2), 129.9 (d, *J*: 2.92 Hz, 4FPh C_1), 117.5 (d, *J*: 23.05 Hz, 4FPh C_3), 112.2 (furan C_3), 112.0 (furan C_4), 39.0 (N-$\underline{C}H_2$-CH_3), 34.6 (-S$\underline{C}H_2$), 14.9 (N-CH_2-$\underline{C}H_3$).

N-(Propyl)-2-{[(4-(4-fluorophenyl)-5-(furan-2-yl)-4H-1,2,4-triazol-3-yl)sulfanyl]acetyl}hydrazine-1-carbothioamide (5g)

White powder, mp 168-169 °C, yield: 80.19%, Anal. Calcd. for: $C_{18}H_{19}FN_6O_2S_2$, C, 49.76; H, 4.41; N, 19.34%; Found: C, 49.39; H, 4.540; N, 19.26%. FT-IR υmax (cm^{-1}): 3317 (N-H), 3149, 3086 (ar. C-H), 2962, 2930, 2862 (al. C-H), 1704 (C=O). ^{1}H-NMR (500 MHz) (DMSO-d_6) δ (ppm): 10.22 (s, 1H, CONH), 9.32 (s, 1H, NH), 8.21 (s, 1H, NH), 7,80 (d, *J*: 1.7 Hz, 1H, furan C_5-H), 7.65-7.58 (m, 2H, 4FPh$C_{2,6}$-H), 7.50 (t, *J*: 8.69 Hz, 2H, 4FPh$C_{3,5}$-H), 6.55 (dd, *J*: 3.5 ; 1.7 Hz, 1H, furan C_4-H), 6,24 (d, *J*: 3.5 Hz, 1H, furan C_3-H), 3.90 (s, 2H, S$C\underline{H}_2$), 3.46 (m, 2H, N-$C\underline{H}_2$-CH_2-CH_3), 1.56 (h, *J*: 7.4 Hz, 2H, -CH_2-$C\underline{H}_2$-CH_3), 0.83 (t, *J*: 7.4 Hz, 3H, -CH_2-CH_2-$C\underline{H}_3$). ^{13}C-NMR (APT) (125 MHz) (DMSO-d_6) δ (ppm): 181.7 (C=S), 167.0 (C=O), 163.3 (d, *J*: 148.95 Hz; 4FPh C_4), 152.1 (triazole C_2), 147.9 (triazole C_5), 145.5 (furan C_5), 141.1 (furan C_2), 130.6 (d, *J*: 9.25 Hz, 4FPh C_2), 129,8 (d, *J*: 2.69 Hz, 4FPh C_1), 117.5 (d, *J*: 23.36 Hz; 4FPh C_3), 112.2 (furan C_3), 112.0 (furan C_4), 45.7 (N-$\underline{C}H_2$-CH_2-CH_3), 34.5 (-S$\underline{C}H_2$), 22.4 (N-CH_2-$\underline{C}H_2$-CH_3), 11.5 (N-CH_2-CH_2-$\underline{C}H_3$).

N-(Butyl)-2-{[(4-(4-fluorophenyl)-5-(furan-2-yl)-4H-1,2,4-triazol-3-yl)sulfanyl]acetyl}hydrazine-1-carbothioamide (5h)

White powder, mp 197 °C, yield: 74.46%, Anal. Calcd. for: $C_{19}H_{21}FN_6O_2S_2$, C, 50.88; H, 4.72; N, 18.74%; Found: C, 50.71; H, 4.942; N, 18.70%. FT-IR υmax (cm^{-1}): 3340 (N-H), 3163, 3095 (ar. C-H), 2955, 2933, 2872 (al. C-H), 1712 (C=O). 1H-NMR (500 MHz) (DMSO-d_6) δ (ppm): 10.22 (s, 1H, NH); 9.33 (s, 1H, NH); 8.18

(s, 1H, NH); 7.79 (d, *J*: 1.15 Hz, 1H, furan C_5-H); 7.66-7.59 (m, 2H, 4FPh$C_{2,6}$-H); 7.49 (t, *J*: 8.7 Hz, 2H, 4FPh$C_{3,5}$-H); 6.54 (dd, *J*: 3.5; 1.8 Hz, 1H, furan C_4-H); 6.25 (d, *J*: 3.5 Hz, 1H, furan C_3-H); 3.91 (s, 2H, SC$\underline{H}_2$); 3.51 (q, *J*: 7.4 Hz, 2H, N-C$\underline{H}_2$-CH_2-CH_2-CH_3); 1.54 (p, *J*: 7.4 Hz, 2H, N-CH_2-C$\underline{H}_2$-CH_2-CH_3); 1.27 (h, *J*: 7.4 Hz, 2H, N-CH_2-CH_2-C$\underline{H}_2$-CH_3); 0.87 (t, *J*: 7.4 Hz, 3H, N-CH_2-CH_2-CH_2-C$\underline{H}_3$). ^{13}C-NMR (APT) (125 MHz) (DMSO-d_6) δ (ppm): 181.8 (C=S); 167.0 (C=O); 163.3 (d, *J*: 248.62 Hz, 4FPh C_4); 152.1 (triazole C_2); 147.9 (triazole C_5); 145.5 (furan C_5); 141.1 (furan C_2); 130.6 (d, *J*: 9.19 Hz, 4FPh C_2); 129.8 (d, *J*: 2.93 Hz, 4FPh C_1); 117.5 (d, *J*: 23.28 Hz; 4FPh C_3); 112.2 (furan C_3); 112.0 (furan C_4); 43.8 (N-C$\underline{H}_2$-CH_2-CH_2-CH_3), 34.5 (-SC$\underline{H}_2$), 31.3 (N-CH_2-C$\underline{H}_2$-CH_2-CH_3), 19.8 (N-CH_2-CH_2-C$\underline{H}_2$-CH_3), 14.2 (N-CH_2-CH_2-CH_2-C$\underline{H}_3$).

N-(Allyl)-2-{[(4-(4-fluorophenyl)-5-(furan-2-yl)-4H-1,2,4-triazol-3-yl)sulfanyl]acetyl}hydrazine-1-carbothioamide (5i)

White powder, mp 151-152 °C, yield: 69.76%, Anal. Calcd. for: $C_{18}H_{17}FN_6O_2S_2$, C, 49.99; H, 3.96; N, 19.43%; Found: C, 49.50; H, 4.063; N, 19.31%. FT-IR υmax (cm^{-1}): 3316 (N-H), 3156, 3083, 3012 (ar. C-H), 2921, 2855 (al. C-H), 1705 (C=O). ^{1}H-NMR (500 MHz) (DMSO-d_6) δ (ppm): 10.28 (s, 1H, CONH), 9.44 (s, 1H, NH), 8.37 (s, 1H, NH), 7.79 (d, *J*: 1.80 Hz, 1H, furan C_5-H), 7.65-7.58 (m, 2H, 4FPh$C_{2,6}$-H), 7.48 (t, *J*: 8.7 Hz, 2H, 4FPh$C_{3,5}$-H), 6.54 (dd, *J*: 3.53; 1.80 Hz, 1H, furan C_4-H), 6.24 (d, *J*: 3.53 Hz, 1H, furan C_3-H), 5.85 (ddt, *J*: 17.20; 10.30; 5.3 Hz, 1H, -NCH_2C$\underline{H}$=CH_2), 5.13 (dd, *J*: 17.20; 1.70 Hz, 1H, -NCH_2CH=C$\underline{H}_2$), 5.02 (dd, J: 10.30; 1.7 Hz, 1H, -NCH_2CH=C$\underline{H}_2$), 4.17 (s, 2H,-NC$\underline{H}_2$CH=CH_2), 3.95 (s, 2H, SC$\underline{H}_2$). ^{13}C-NMR (APT) (125 MHz) (DMSO-d_6) δ (ppm): 182.1 (C=S), 167.1 (C=O), 163.3 (d, *J*: 148 Hz; 4FPh C_4), 152.1 (triazole C_2), 147.9 (triazole C_5), 145.5 (furan C_5), 141.1 (furan C_2), 135.2 (-NCH_2CH=CH_2), 130.6 (d, *J*: 9.25 Hz, 4FPh C_2), 129,8 (d, *J*: 2.69 Hz, 4FPh C_1), 117.5 (d, *J*: 23.36 Hz; 4FPh C_3), 115.7 (-NCH_2CH=CH_2), 112.2 (furan C_3), 112.0 (furan C_4), 46.3 (-NCH_2CH=CH_2), 34.6 (-SC$\underline{H}_2$).

Antibacterial activity studies

The antimicrobial activity of a series of thiosemicarbazides bearing 1,2,4-triazole **(5a-i)** was investigated against *K. pneumoniae* (BAA-2146), *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), *E. faecalis* (ATCC 29212), and *P. aeruginosa* (ATCC 27853). Both disk diffusion and broth microdilution methods were employed to assess activity, ensuring consistency across experiments.[26,27]

For the broth microdilution method, a stock solution of each compound was prepared at a concentration of 100 micromolar (μM), followed by serial dilutions in sterile 96-well microplates. Muller, Muller-Hinton broth was added to each well, and bacterial suspensions were adjusted to a 0.5 McFarland standard. The plates were incubated at 35-37 °C for 18-24 hours under appropriate conditions, and the minimum inhibitory concentrations (MICs) were determined manually.

In the disk diffusion assay, blank antibiotic disks were impregnated with the compounds, and the zone diameters of inhibition were measured after incubation. The tests were standardized with DMSO as a negative control and specific antibiotics as positive controls: Vancomycin (20 μM) for *S. aureus* and *E. faecalis*, and Meropenem (78 μM) for *P. aeruginosa*. All experiments were conducted following European Committee on Antimicrobial Susceptibility Testing guidelines, and bacterial strains were maintained at -80 °C until use.

Statistical analysis

The antimicrobial assay results were expressed in μM units to enable reliable comparison among the synthesized derivatives and reference compounds. The obtained data were descriptively evaluated, with MIC values serving as the primary measure of antimicrobial potency. This ensured a consistent and reproducible framework for interpreting the biological activity across all tested compounds. No further statistical testing was applied.

RESULTS

Chemistry

All designed novel thiosemicarbazide compounds (**5a-i**) were obtained according to the methods depicted in the experimental section. The chemical pathway shown in Scheme 1 outlines a synthesis process that involves five steps. Furan-2-carbohydrazide and 4-fluorophenylisothiocyanate were boiled under reflux in ethanol to yield N-(4-Fluorophenyl)-2-(furan-2-carbonyl)hydrazine-1-carbothioamide (**1**). Solution of (**1**) in NaOH (2N) was heated under reflux for 4 h, then it was neutralized with 12.5% HCl to yield 4-(4-Fluorophenyl)-5-(furan-2-yl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**2**). A mixture of (**2**), K_2CO_3 and $BrCH_2COOEt$ was refluxed in acetone to obtain Ethyl ((4-Fluorophenyl)-5-(furan-2-yl)-*4H*-1,2,4-triazole-3-yl) sulfanyl)acetate (**3**). Compound 3 and $NH_2NH_2.H_2O$ were heated under reflux in ethanol to yield 2-[[4-(4-Fluorophenyl)-5-(furan-2-yl)-*4H*-1,2,4-triazol-3-yl]sulfanyl]acetohydrazide (**4**). A solution of 4 and the appropriate isothiocyanate in absolute ethanol was heated under reflux to yield **5a-i**. The designed compounds (**5a-i**) were synthesized with good yields (61.10-82.66%).

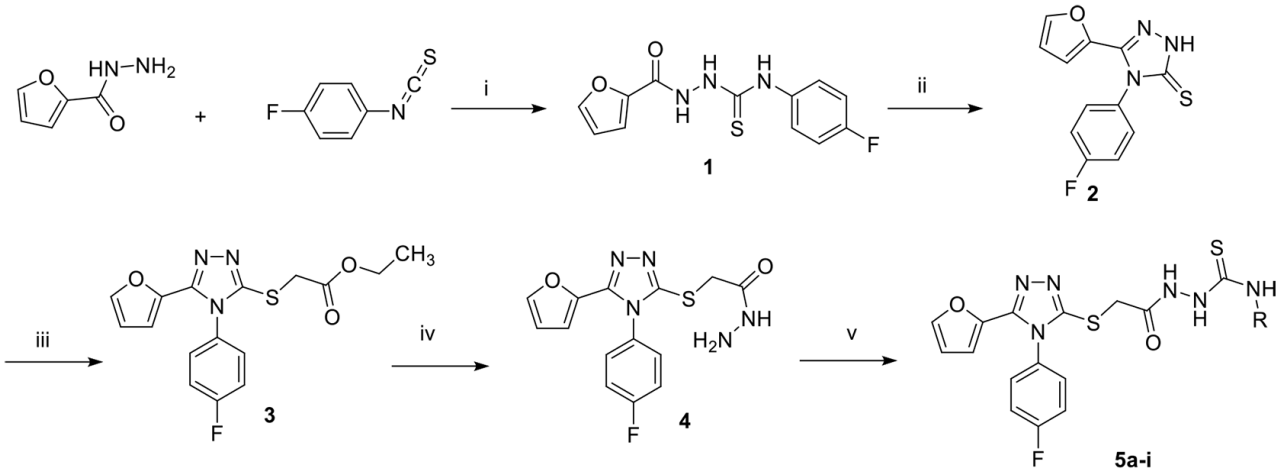
Antimicrobial activity

According to the *in vitro* assay results, against *P. aeruginosa* compound **5g** showed the lowest MIC (<0.78 μM), at least 100-fold more potent than Meropenem (MIC = 78 μM), while **5i** (MIC = 1.56 μM) was ~50-fold more potent. For *S. aureus*, **5e** (MIC = 12.5 μM) outperformed Vancomycin (MIC = 20 μM), and **5b** and **5h** (MIC = 25 μM) were comparable to Vancomycin. No compound was active (MIC >100 μM) against *E. coli*, *K. pneumoniae*, or *E. faecalis*, as can be seen in Table 1.

DISCUSSION

IR spectra provide a straightforward diagnostic of the hydrazide-to-thiosemicarbazide transformation: in compound **4**, the terminal -NH_2 group exhibits the characteristic pair of N-H stretching bands, whereas in **5a-i** these bands disappear and are replaced by a single N-H stretch consistent with conversion of -NH_2 to a secondary -NH- within the thiosemicarbazide framework.[28]

Scheme 1. Synthesis pathway of the titled compounds (5a-i) Reagent and conditions: *i:* EtOH, *ii:* 2N NaOH, 12.5% HCl, *iii:* α-BrCH2COOC2H5/K2CO3/acetone, *iv:* NH2NH2.H2O/EtOH, *v:* RNCS/EtOH



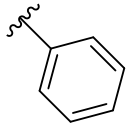
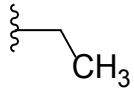
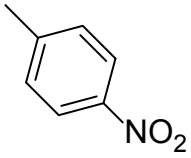
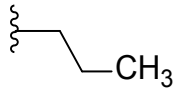
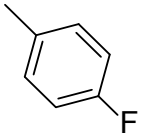
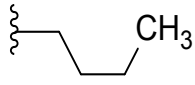
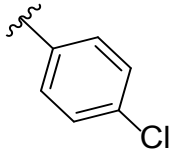
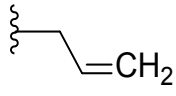
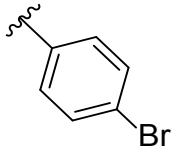
Compound	R	Compound	R
5a		5f	
5b		5g	
5c		5h	
5d		5i	
5e		-	-

Table 1. MICs of thiosemicarbazide derivatives (5a-i) against various microorganisms

Compound	Microorganisms				
	Escherichia coli	Pseudomonas aeruginosa	Klebsiella pneumoniae	Staphylococcus aureus	Enterococcus faecalis
Dimethyl sulfoxide	>100	>100	>100	>100	>100
5a	>100	>100	>100	>100	>100
5b	>100	>100	>100	**25**	>100
5c	>100	>100	>100	>100	>100
5d	>100	>100	>100	>100	>100
5e	>100	>100	>100	**12.5**	>100
5f	>100	>100	>100	>100	>100
5g	>100	**<0.78**	>100	>100	>100
5h	>100	>100	>100	**25**	>100
5i	>100	**1.56**	>100	>100	>100
Vancomycin	-	-	-	20	20
Meropenem	-	**78**	-	-	-

MICs: Minimum inhibitory concentrations

In the ^{1}H-NMR spectra, the terminal NH_2 protons of compound 4, initially observed at 4.32 ppm, disappeared in the thiosemicarbazide derivatives (**5a-i**). However, the NH_2 protons of the newly formed thiosemicarbazide group in **5a-i** appeared in the range of 10.22-8.18 ppm, confirming their formation.[29] Additionally, the aryl protons of the isothiocyanate groups in **5a-i** further supported the confirmation of the structure.

In the ^{13}C (APT) NMR spectra, the carbonyl carbons (-C=O) of compounds **4** and **5a-i** were detected between 166.4 and 167.0 ppm. Carbons associated with the -C=S group in **5a-i**, indicative of the thiosemicarbazide functionality, appeared at 182.1-180.9 ppm.[30] The aryl carbons of the isothiocyanate groups also reinforced the structural integrity of the compounds.

These spectroscopic results (^{1}H NMR, ^{13}C NMR, and FT-IR), along with elemental analysis, unequivocally confirmed the successful transformation of compound four into the thiosemicarbazide derivatives **5a-i**. No significant side reactions were observed, and the target compounds were obtained in moderate to good yields, with all data aligning with the assigned structures and literature.[23,24]

Based on our MIC data, we can describe the structure-activity relationship (SAR) in more detail - see Table 1 and Figure 3. Against *P. aeruginosa*, the three-carbon **n-propyl** group on the thiosemicarbazide nitrogen gave the best result (compound **5g**, MIC <0.78 µM), which is more than 100-fold better than Meropenem (MIC 78 µM). When **n-propyl** was changed to **allyl**, the activity dropped about two-fold (compound **5i**, 1.56 µM). Shorter (**ethyl**) or longer (**n-butyl**) chains were inactive (MIC >100 µM), and replacing the aliphatic chain with an **aryl** (phenyl) group also removed the activity. These trends suggest that *P. aeruginosa* needs a simple, three-carbon saturated chain at this position, probably because it gives the right size and shape for the target and also helps the compound pass the outer membrane; adding an aromatic ring may be too bulky or too rigid, and changing to an allyl group may change the geometry or polarity in a way that weakens binding.

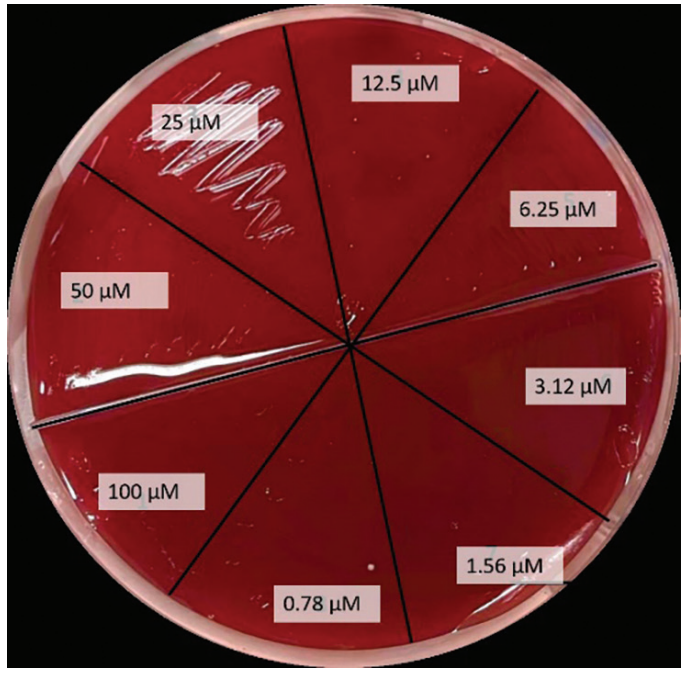


Figure 3. No inhibition toward the growth of *Pseudomonas aeruginosa* (ATCC 27853) at different concentrations of the compound 5 g

Against *S. aureus*, activity increased when the para substituent on the phenyl ring was **less electronegative**: ***p*-Br** (5e, 12.5 µM) was stronger than ***p*-NO_2** (5b, 25 µM), while ***p*-F** and ***p*-Cl** were inactive (MIC >100 µM). One simple explanation is that

bromine is less electronegative and more polarizable, so it may improve hydrophobic contacts in the binding site, while very electron-withdrawing or very small substituents reduce those contacts. In the alkyl series for *S. aureus*, only **n-butyl** (5h, 25 μM) was active; **ethyl**, **n-propyl**, and **allyl** analogs were inactive, which may indicate that a slightly longer hydrophobic tail helps interaction with the Gram-positive cell envelope or with the target pocket.

None of the compounds were active against *E. coli, K. pneumoniae*, or *E. faecalis* (MIC >100 μM). The reason is not clear at this stage; common possibilities include poor permeability, strong efflux, or a different/absent target in these organisms. In future work we plan to check simple permeability and efflux effects and do basic target-engagement tests to understand these findings and to guide the design of more active analogs (for example, small changes around **n-propyl** for *P. aeruginosa* and around **p-Br** for *S. aureus*).

Upon evaluating all data thoroughly, to clarify the SAR key observations and insights are given as follows, see in Table 1 and Figure 3.

1. The three-carbon alkyl chain (n-propyl, compound **3g**) is optimal for activity against *Pseudomonas aeruginosa*. Shorter (ethyl, compound **3f**) or longer (n-butyl, compound **3h**) chains are inactive
2. Unsaturation and aryl substitution reduce activity against *P. aeruginosa*.
   - n-propyl > allyl (≈2× loss in potency when changing to allyl)
   - n-propyl > allyl > phenyl (aryl) (phenyl = inactive)
3. Less-electronegative para substituents improve activity against *Staphylococcus aureus*.
   - *p*-Br > *p*-NO_2, while *p*-F and *p*-Cl are inactive
   - Among alkyls, only n-butyl shows activity

CONCLUSION

There is still a significant danger arising from various bacteria today. Because many bacterial types, such as *S. aureus* and *P. aeruginosa*, are quickly resistant to the current antibiotics, the design and evaluation of novel potent antimicrobial molecules are vital. In this study, nine novel thiosemicarbazide-bearing 1,2,4-triazole compounds were synthesized using simple and practical methods, and their structure was characterized using methods such as FT-IR, ^{1}H-NMR, ^{13}C-NMR, elemental analysis, and mass spectroscopy. All synthesized compounds were investigated for their antibacterial activities against diverse bacteria including *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus*, and *E. faecalis*. Especially the compounds carrying 4-bromophenyl substitution at the N position of the thiosemicarbazide nucleus ring showed high antibacterial activities against *S. aureus* with 12.5 μM, whereas the n-propyl substituted derivative displayed the most activity against *P. aeruginosa* with 0.78 μM. Consequently, we believe that the biological assay data and SAR evaluation obtained from this study may assist in the future discovery of new and more potent antimicrobial compounds.

Ethics

Ethics Committee Approval: Not required.

Informed Consent: Not required.

Footnotes

Authorship Contributions

Concept: E.D.D., E.D.K., H.Ö.D., F.B., E.G., N.U.G., Design: E.D.D., E.D.K., H.Ö.D., F.B., E.G., N.U.G., Data Collection or Processing: E.D.D., E.D.K., H.Ö.D., F.B., E.G., N.U.G., Analysis or Interpretation: E.D.D., E.D.K., H.Ö.D., F.B., E.G., N.U.G., Literature Search: E.D.D., E.D.K., H.Ö.D., F.B., E.G., N.U.G., Writing: E.D.D., E.D.K., H.Ö.D., F.B., E.G., N.U.G.

Conflict of Interest: The authors declare no conflicts of interest.

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ORIGINAL ARTICLE

Dissolution Enhancement of Lycopene Compacts by Liquisolid Technique

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ABSTRACT

Objectives: Lycopene is a powerful antioxidant with diverse health benefits. However, it belongs to the Biopharmaceutics Classification System II; thus, it depicts poor water solubility and dissolution. Its lipophilic nature hinders the bioavailability of this drug. To overcome these limitations, namely, poor solubility and bioavailability, several approaches have been tried so far, such as co-solvency, size reduction or micronization, complexation, adsorption on high surface area carriers, *etc.* The present research aimed to apply the liquisolid technique to prepare lycopene liquisolid compacts with an improved dissolution profile. The impact of parameters such as carrier and drug concentration percentage on drug dissolution was evaluated in liquisolid compacts.

Materials and Methods: Lycopene was extracted by Soxhlet extractionand then characterized by ultraviolet spectroscopy, infrared spectroscopy, thin-layer chromatography, and melting point. Liquisolid compacts of lycopene were formulated by using excipients such as non-volatile solvent (glycerine), carrier (Avicel PH 101, Fujicalin, Neusilin US2), disintegrant (Croscarmellose sodium), and diluent (lactose). The different formulation batches of liquisolid compacts were formulated and evaluated based on different pre-compression and post-compression parameters.

Results: Powder X-ray Diffraction (PXRD) and Fourier transform infrared spectroscopy were utilized to analyze drug-excipient interaction; these studies showed no evidence of any physical or chemical interaction between the drug(s) and the excipients. The PXRD of lycopene showed sharp and intense peaks at diffraction angles (2θ) such as 12.563, 19.176, 19.636, 20.062, 21.283, 26.629, 29.479, 30.235, and 39.997, which indicates a crystallinestructure. The PXRD of the physical mixture of lycopene and excipients showed similar sharp peaks (12.582, 19.202, 19.634, 20.045, 21.304, 26.565, 29.474, 30.250, and 40.065), indicating thatthere is no drug-excipient interaction occurring. Lycopene was extracted and characterized. IR spectroscopy and PXRD showed no drug-excipient interaction. The lycopene liquisolid compacts passed both pre-compression and post-compression evaluations within acceptable limits.

Conclusion: The formulation batch F-7, formulated with Neusilin US2 as a carrier and 40% drug concentrationshowed 98% *in vitro* drug release and thus it was selected as the optimized formulation with improved dissolution.

Keywords: Lycopene, Biopharmaceutics Classification SystemII, dissolution, liquisolid compacts, extraction, Neusilin US2

INTRODUCTION

The low solubility and dissolution of a drug are a leading hurdle for the formulation scientist. Nearly 40% of recently discovered drugs are water-insoluble.[1] Such lipophilic drugs belong to the Biopharmaceutics Classification System II (BCS II). Many approaches to enhance dissolution of water-insoluble drugs are available, such as solid dispersion, micronization, liposomes, solid lipid nanoparticles, nanosuspensions, hydrotropy, *etc*.[2-6] Poor solubility, release kinetics, dissolution rate, and photolability are prominent problems associated with most drugs. The liquisolid technique has emerged as an important approach for overcoming these challenges.[7]

This technique involves converting liquid medication (drug solubilized in a non-volatile solvent) into free-flowing, dry, and compressible powder by the addition of calculated quantities of carrier and coating material. In this technique, the drug is first solubilized in a non-volatile solvent with maximum solubility. Then, this solution is incorporated into the carrier for its absorption.[8] The adsorption of the liquid medication takes place onto the surface of the carrier particles. Next,

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coating material is added, which adsorbs liquid medication to form free-flowing, dry, and compressible powder. Afterwards, a disintegrant is added to the powder, and then it is directly compressed to form liquisolid compacts.[9] Four mechanisms are proposed for enhanced dissolution exhibited by the liquisolid compacts-increased drug surface area,[10] increased drug water solubility,[11] increased wetting ability,[12] and enhanced porosity.[13] The enhanced dissolution improves bioavailability and biological activity of the drug.[14]

Lycopene is a powerful antioxidant thatbelongs to the carotenoid family. It has 100 times greater free radical quenching properties than vitamin E. It is a redpigment, which gives colour to both fruits and vegetables. Lycopene, known for its antioxidant properties, has been found to reduce oxidative stress, a significant contributor to the development of metabolic diseases.[15] Due to its antioxidant properties, it has exhibited diverse health benefits. It has shown protective effects against many chronic diseases *in vitro* and *in vivo* studies.[16] It has demonstrated beneficial effects in cancer, diabetes, inflammatory diseases, cardiovascular diseases, skin damage, male infertility, osteoporosis, *etc*.[17]

Lycopene is completely water-insoluble in nature, leading to a major formulation problem. It has good penetration because of its lipophilic nature, hence it belongs to BCS II. Due to its low solubility, it has exhibited a poor dissolution rate.[18] This leads to low bioavailability and biological activity of the drug.[19] During the literature search, it was discovered that various nanotechnology approaches had been applied to lycopene to enhance its dissolution and improve its biological activity, such as polyethylene glycol (PEG) nanoparticles,[20] nanoliposomes,[21] inorganic nanoparticles,[22] nano-niosomes,[23] nano-structured lipid carriers,[24] Self-Microemulsifying Drug Delivery System,[25] microemulsions,[26] transfersomes,[27] ethosomes,[28] phytosomes,[29] nanocapsules,[30] carbon nanotubes,[31] polymeric nanoparticles,[32] *etc*. These nanoformulations have high production costs, stability issues, and toxicity problems, which hinder the commercialization of such formulations.[32-34]

The liquisolid technique is a recent and innovative approach for the enhancement of dissolution of lipophilic drugs, and it overcomes the drawbacks of the previously mentioned approaches. Its cheaper excipients, low cost of production, convenient manufacturing, good compressibility, and flowing property make it feasible for industrial large-scale production.[9]

The aim of this study was to prepare and evaluate lycopene-based liquisolid compacts and select the optimized formulation with improved dissolution.

MATERIALS AND METHODS

Materials

Ethyl acetate was procured from Pure Chemicals Co., Chennai. At the same time, Hexane and Acetone were obtained from Advent Chembio Pvt. Ltd., Mumbai, Monobasic potassium phosphate was purchased from Sigma-Aldrich Chemicals Pvt. Ltd., Bangalore, India, PEG 200,400 were obtained from Research-Lab Fine Chem Industries, Mumbai, India, and tween 20 and 80 were procured from Molychem, Mumbai, India, and Neusilin US2 was obtained from Ottokemi, Waliv, India.

Extraction of lycopene

Lycopene was extracted using the Soxhlet method. About 100 g of tomato was dried at 60 °C for 24 hours and thenpowdered. The dried powder was extracted using 150 mL ofethyl acetate for around 12 hours in a Soxhlet apparatus. The extract obtained was concentrated using a rotary evaporator, and then a few drops of methanol were added to precipitate out lycopene. The precipitates formed were dried and then weighed.[35]

Characterization of lycopene

IR spectroscopy

The IR spectrum of the extracted lycopene was assessed using a Bruker FTIR spectrophotometer (Alpha, Bruker, America) in the range of 4000 to 400 cm^{-1} wavelength. The IR spectral analysis was performed by mixing 5 mg of the sample with 100 mg of potassium bromide, which was subjected to a pressure of 12000 psi for about 3 min. The characteristic peaks found in the IR spectrum wereutilized to know the functional groups which are present in the spectrum. This spectrum was compared with the IR spectrum of standard lycopene for its authentication purposes.[36]

Thin-layer chromatography

Using a capillary tube, the extracted lycopene was applied to the pre-coated thin-layer chromatography (TLC) plate. This plate was kept in a developing chamber containing a mixture of hexane and acetone (70:30), which was used as the mobile phase. After the completion of the development of the TLC plate, the R_f values of the standard and extracted lycopene were contrasted. The following formula can be used to determine theR_f value:

$$R_f = \frac{\textit{Distance travelled by solute}}{\textit{Distance travelled by solvent}}$$

Melting point

The melting point determination of extracted lycopene was performed on the capillary melting point apparatus. A small amount of the sample was placed in a thin-walled capillary tube closed at one end. The capillary was then placed into the heated chamber, and the temperature was noted when the substance became completely transparent. This is considered to be the melting point.[37]

Pre-formulation studies

IR spectroscopy

The IR spectra of lycopene, Avicel PH 101, fujicalin, Neusilin US2, aerosil 200, crosscarmellose, lactose, and optimized formulation were recorded on a Bruker FTIR spectrophotometer (Alpha, Bruker) in the range of 4000 to 400 cm^{-1} wavelength to detect any drug-excipient interaction. In this analysis, IR spectral analysis was performed by mixing 5 mg of the sample with 100 mg of potassium bromide, which was pressed at 12000 psi for about 3 min.[14]

Powder X-ray diffraction (PXRD)

The PXRD pattern of lycopene, Avicel PH 101, fujicalin, neusilin US2, aerosil 200, crosscarmellose, lactose, and physical mixture was recorded on an X-ray diffractometer (Bruker Corporation, America) using Ni-filtered CuKα radiation with 1.540 Å wavelength. Data were scanned from the 10-80 ˚ 2θ range.[38]

Saturation solubility studies

To select the best non-volatile solvent, saturation solubility studies are conducted. The drug was dissolved in non-volatile solvents such as propylene glycol, glycerine, PEG 200, PEG 400, tween 20, tween 80, and distilled water. The excess quantity of the drug was dissolved in the above-mentioned non-volatile solvents. The saturated solutions formed were shaken on the water bath shaker apparatus for 48 hours at 25 ˚C at constant vibration. After the saturated solutions were kept under constant vibration, they were filtered and diluted with phosphate buffer pH 7.2. The diluted solution was analyzed by a ultraviolet-visible (UV-Vis) spectrophotometer at 363 nm. The absorbance was determined three times for each solution to calculate the solubility of lycopene.

Solubility enhancement analysis

The prepared and selected liquisolid powder was evaluated for solubility enhancement. In this, an excess quantity of liquisolid powder was dissolved in the best non-volatile solvent (glycerine). The saturated solutions formed were shaken on the water bath shaker apparatus for 48 hours at 25 ˚C at constant vibration. After saturated solutions were kept under constant vibration, it was filtered and diluted with phosphate buffer pH 7.2. The diluted solution was analyzed by a UV-Vis spectrophotometer at 363 nm. The absorbance was determined three times for each solution to evaluate solubility enhancement.[39]

Angle of slide determination

Firstly, the drug was solubilized in a non-volatile solvent in different concentrations. A binary mixture of carrier and coating material was prepared in a ratio of 20:1. Powder mixtures were formed by mixing an increasing amount of the binary mixture into the drug solution. Each powder mixture was placed on a polished metal plate, and then the metal plate was tilted till the powder started to slide. The angle (formed between the plate and the horizontal plane) at which the powder slides were noted is termedthe angle of slide. The powder mixture with a slide angle of 33 ˚ was selected for formulation preparation.[40]

Preparation of liquisolid powder

The required quantity of drug (20 mg) was weighed and dissolved with calculated quantities of non-volatile solvent (glycerine) to different drug concentration solutions (40%, 50%, and 60% *w/w*). Different carriers, i.e., Avicel PH 101 (microcrystalline cellulose), Fujicalin (dibasic calcium phosphate), and Neusilin US2 (aluminometasilicate), were selectedto select the best carrier for liqui-solid compacts. Aerosil 200 was selected as a coating material in all formulation batches. The excipient ratio was kept at a constant value of 20, as it is regarded as optimum.[41] The required quantities of the carrier and coating material were blended with the drug solution in the mortar and pestle. Crosscarmellose (5% concentration) and lactose (diluent) were mixed properly to obtain a liquisolid powder.[42] Table 1 depicts the preparation composition of all liquisolid batches.

Pre-compression evaluation of the liquisolidpowder

The pre-compression properties of the liquisolid powder are determined usingthe following parameters:

Angle of repose: This is the maximum angle possible between the surface of a pile of powder and the horizontal plane. Ten grams of powder was allowed to flow throughthe funnel from a height of 4 cm abovethe base. The height of the pile anddiameter of the base were measured, and the angle of repose was calculated using the following formula:

tan θ = h/rθ = tan-1 h/r, where = angle of repose, h = height of the heap, r = radius of the heap

Table 1. Preparation of the composition of all liquisolid batches

Group	Batchnumber	Drugconc %	W (mg)	Q (mg)	q (mg)	L_f	CCS (mg)	LSC (mg)	Lactose (mg) (Diluent)	Total weight (mg)
Group I (Avicelp H101)	F1	40	30	119.047	5.952	0.252	8.749	183.748	16.252	200
	F2	50	20	95.238	4.761	0.21	6.999	146.998	53.002	200
	F3	60	13.33	71.428	3.71	0.186	5.423	113.891	86.109	200
Group II (Fujicalin)	F4	40	30	71.428	3.571	0.42	6.249	131.248	68.752	200
	F5	50	20	57.142	2.857	0.35	4.999	104.998	95.002	200
	F6	60	13.33	47.619	2.38	0.279	4.166	87.495	112.505	200
Group III (Neusilin US2)	F7	40	30	23.809	1.19	1.26	3.749	78.748	121.252	200
	F8	50	20	19.047	0.952	1.05	2.999	62.998	137.002	200
	F9	60	13.33	14.285	0.714	0.933	2.416	50.745	149.255	200

W: Non-volatile solvent (glycerine), Q: Carrier (Avicel PH 101, Fujicalin, Neusilin US2), q:Coating material (Aerosil 200), L_f: Liquid loading factor, CCS: Crosscarmellose sodium, LSC: Liquisolid compacts weight

Bulk density: An accurately weighed quantity of powder, which was previously passed through sieve #40 United States Pharmacopeia (USP) and carefully poured into a graduated cylinder. Then, after pouring the powder into the graduated cylinder,the powder bed was made uniform without disturbing it. Then thevolume was measured directly from the graduation marks on thecylinder as mL. The volume measured was calledthe bulk volume, and the bulk density is calculated by the following formula:

bulk density = weight of powder/bulk volume

Tapped density: After measuring the bulk volume, the same measuring cylinder was setinto the tappeddensity apparatus. The tap density apparatus was set to 300 taps per minute and operated for 500 taps. Volume was noted as [V_a] and again tapped for 750 times, and volume was noted as [V_b]. If the difference between V_a and V_b is not greater than 2% then V_b is considered as the final tapped volume. The tapped density is calculated by the following formula:

tapped density = weight of powder / tapped volume

Carr's Index is one of the most important parameters to characterize the nature of powders and granules. It can be calculated from the following equation:

*Carr's Index = (tapped density - bulk density) / tapped density * 100.*

Hausner's Ratio: Hausner's ratio is an important characteristic for determining the flow property of powder and granules. This can be calculated by thefollowing formula:

Hausner's Ratio = tappeddensity / bulkdensity[43-46]

Compression

The different formulation batches of liquisolid powder were compacted into tablets by using an eight-station rotary compression machine with an 8 mm punch size, while the compression force was adjusted to get an acceptable hardness of tablets.[47]

Post-compression evaluation of the liquisolid compacts

The post-compression properties of the liquisolid compacts are determined by using the following parameters: thickness, weight variation, hardness, friability, disintegration test, and content uniformity.

In vitro release [48-53]

Mathematical modeling

The percentage cumulative quantity of the drug released from the optimized formulation batch at various time intervals was fitted into different mathematical models of drug release profile, such as Zero Order Model, Higuchi Model, First Order Model, Korsmeyer-Peppas Model, and Hixon-Crowell Model,to characterize the drug release mechanism.[54-57] The importance of *in vitro release* data in drug product development has been significant. The release kinetics can be influenced by the drug type, polymorphic form, solubility, and content proportion in the pharmaceutical dosage form. The data were fitted into various release kinetics equations, and the drug release rate was calculated. The suitability of an equation is judged forthe foremost equation using the correlation coefficient between percent drug release versus time.

RESULTS

Extraction of Lycopene

The Soxhlet extraction was conductedto extract lycopene from tomato using ethyl acetate as a solvent. The result showed that the amount of lycopene extracted from this method was 4.58±0.007 mg/g of dried matter.

Characterization of Lycopene

Infrared spectroscopy

IR spectra of standard and extracted lycopene are shown in Figures 1 and 2. The characteristic peaks of lycopene were found such as 1665.61 C=C (stretch); 1475.45 C-H (stretch); 1081.97 C-H (trans); and 717.88 C-H (out of plane). The characteristic peaks of standard lycopene were found such as 1664.46 C=C (stretch); 1476.28 C-H (stretch); 1001.69 C-H (trans); and 717.88 C-H (out of plane). While other peaks are present due to extraction solvent or minute quantities of impurity substances, the main peaks were identified as the target compounds.

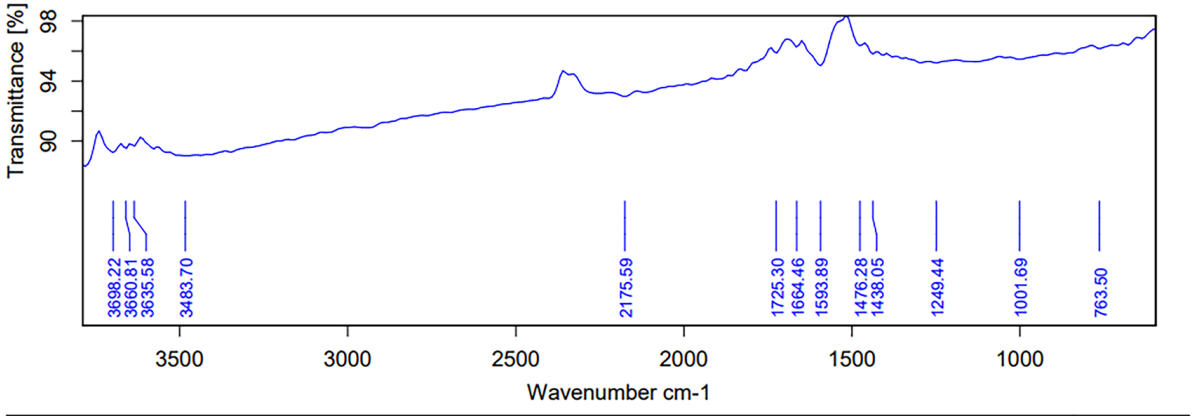

Figure 1. IR spectrum of standard

IR: Infrared

TLC

R_f value for standard lycopene and extracted lycopene was found to be the same, *i.e.*, 0.85 that is performed in pre-coated TLC plate.

Melting point

The melting point determination of extracted lycopene and standard lycopene was carried out using a capillary melting point apparatus. The melting point of extracted lycopene and standard lycopene 171-173 °C and 172 °C.

Pre-formulation studies

FTIR

Figure 3 depicts the stacked ATR of lycopene, excipients, and the optimized formulation. The IR spectrum of lycopene depicts characteristic peaks at 1664.46 cm^{-1} due to C=C (stretch), 1593 cm^{-1} due to C=C (stretch), 1476.28 cm^{-1}, and 1001.69 cm^{-1} due to C-H (trans). The IR spectrum of the physical mixture depicts similar characteristic peaks (1695.05 cm^{-1}, 1647.82 cm^{-1}, 1519.74 cm^{-1}, 1026.63 cm^{-1}), and therefore, there is no drug-excipient interaction occurring.

Saturation solubility studies

The saturation solubility studies for lycopene were conducted in different non-volatile solventssuch as glycerin, PEG 200, PEG 400, tween 20, tween 80, propylene glycol, distilled water, *etc.* Lycopene's solubility in glycerin was found to be 152.35±4.78 mg/mL.

PXRD

Figure 4 depicts the stacked PXRD of lycopene, excipients, and the physical mixture. The PXRD of lycopene showed sharp and intense peaks at diffraction angles (2θ) such as 12.563, 19.176, 19.636, 20.062, 21.283, 26.629, 29.479, 30.235, and 39.997, which indicates a crystallinestructure. The PXRD of the physical mixture of lycopene and excipients showed similar sharp peaks (12.582, 19.202, 19.634, 20.045, 21.304, 26.565, 29.474, 30.250, and 40.065), indicating thatthere is no drug-excipient interaction occurring.

Solubility enhancement analysis

The saturated solution of the liquisolid powder in glycerine was prepared. It was shaken for 48 hours, filtered, and diluted with phosphate buffer pH 7.2. The diluted solution was analyzed by UV Vis spectrophotometer at 363 nm. It was found that the optimized liquisolid powder exhibited solubility of 433.833±8.519 mg/ml in glycerine, and hence, the solubility was enhanced.

Pre-compression parameters evaluation of the liquisolid powder

Table 2 depicts the evaluation of the pre-compression parameters of all formulation batches. The angle of repose

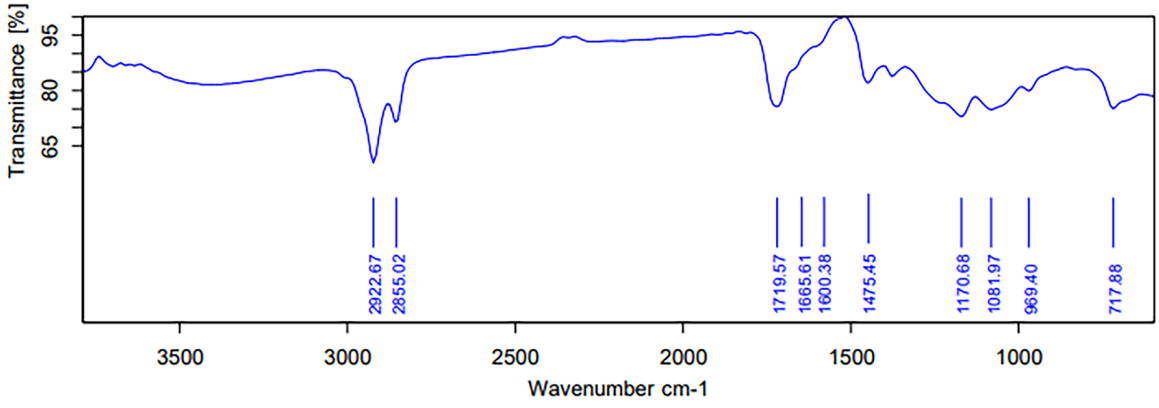

Figure 2. IR spectrum of extracted

IR: Infrared

Table 2. Pre-compression parameters evaluation of the liquisolid powder

Batch number	Angle of repose (θ)	Bulk density (g/mL)	Tapped density (g/mL)	Hausner's Ratio	Carr's Index
F-1	32.799±2.047 °	0.253±0.004	0.277±0.001	1.09±0.025	8.87±1.951
F-2	33.153±5.721 °	0.249±0.005	0.275±0.005	1.10±0.005	9.33±0.382
F-3	34.496±1.829 °	0.253±0.004	0.276±0.004	1.09±0.03	8.17±2.782
F-4	25.249±2.278 °	0.253±0.005	0.278±0.005	1.1±0.017	8.85±1.627
F-5	31.872±1.574 °	0.249±0.005	0.275±0.007	1.10±0.045	9.38±3.773
F-6	28.031±2.539 °	0.254±0.003	0.276±0.004	1.09±0.034	8.05±2.817
F-7	28.94±4.113 °	0.252±0.003	0.275±0.005	1.09±0.034	8.32±2.959
F-8	28.94±4.113 °	0.249±0.003	0.279±0.007	1.12±0.040	10.67±3.400
F-9	26.565±0.00 °	0.250±0.004	0.274±0.002	1.09±0.028	8.60±2.661

of all formulations was found to be in the range of 25.249° to 34.496 °. Bulk density and tapped density of these formulations were within the ranges of 0.249 to 0.254 g/mL and 0.274 to 0.279 g/mL, respectively. Hausner's Ratio of all liquisolid compacts batches varied from 1.09 to 1.12, while Carr's Index was within the range 8.05% to 10.67%. From theseresults, it was concluded that these formulations have excellent to good flow propertiesand thus, they can be utilized to form liquisolid compacts.

Post-compression parameters evaluation of the liquisolid compacts

The thickness of all formulation batches ranges from 3.86 to 4.10 mm. All tablets in each formulation batch showed a % deviation less than 7.5%, so they are within acceptable limits as per USP. The hardness of all formulation batches ranges from 4.166 to 6.166 kg/cm^2. It fulfills the acceptance criteria of not being less than 4 kg/cm^2. The friability of all formulation batches ranges from 0.272 to 0.582%. It was less than 1%; therefore, it fulfills the acceptance criteria as per USP. The disintegration time of all formulation batches ranges from 5.444 to 9.021 min. It was less than 30 min, which is the acceptance criterion for uncoated tablets as per USP. Hence, all formulation batches pass the disintegration test. The % drug content of all formulation batches ranges from 93.467% to 97.036% as depicted in Table 3. It was within the range of85% to 115% according to USP specifications. Hence, all formulation batches fulfill this acceptance criterion.

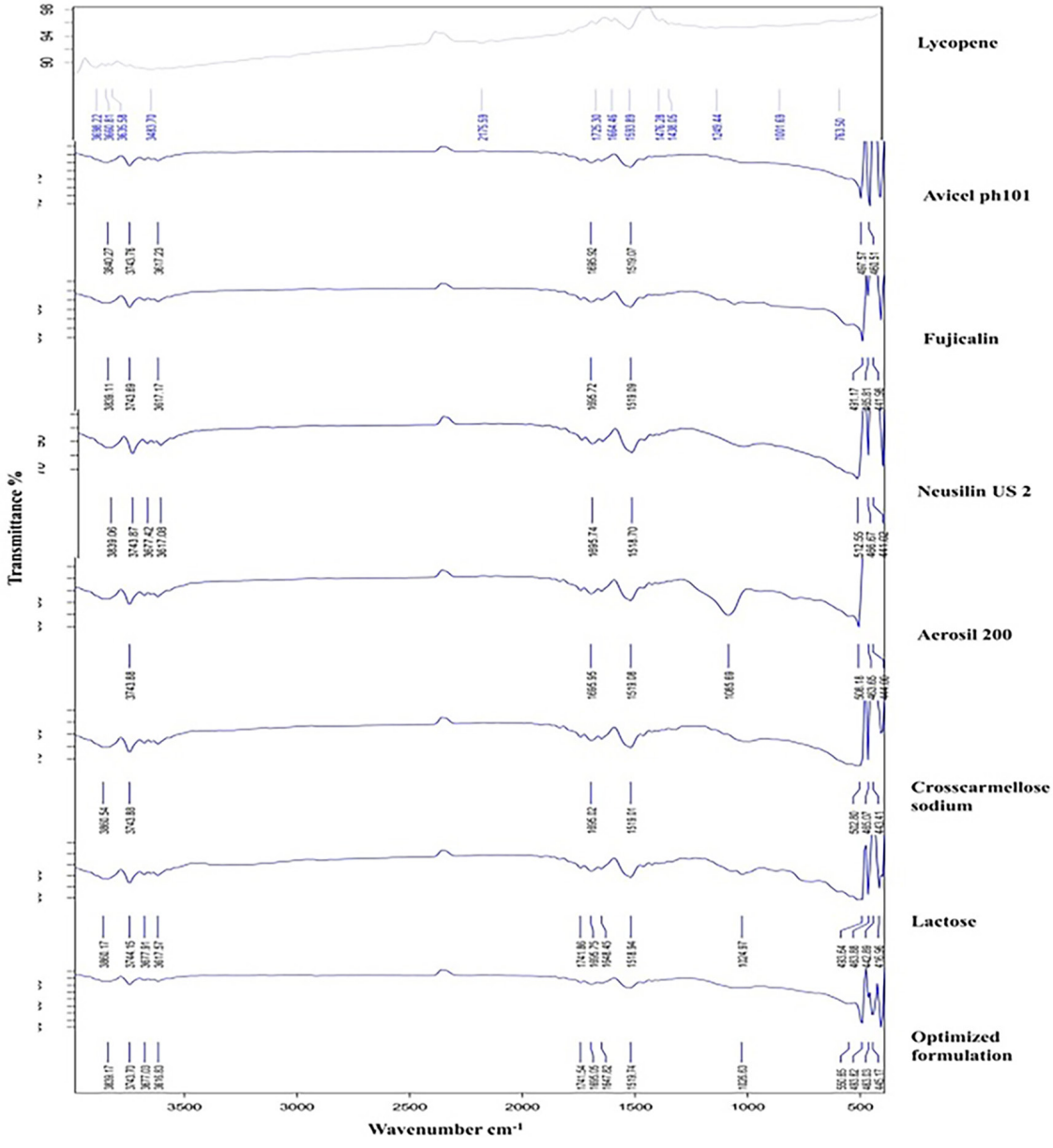

Figure 3. Stacked ATR of Lycopene, excipients and optimized formulation

ATR: Attenuated total reflectance

In vitro release

The *in vitro* drug dissolution study of all formulation batches was carried out by means of a USP type II apparatus with phosphate buffer pH 7.2. Samples for analysis were pipetted out at 5, 10, 15, 30, 45, 60, 90, and 120 min, and the absorbance was measured using a UV-Vis spectrophotometer at 363 nm. The results of an *in vitro* drug release are depicted in Table 3. The two formulation parameters, the effect of the carrier anddrug concentration, that would affect the % cumulative drug release, were investigated in this study.

Formulation batch F-1, F-2, and F-3, which were formulated with AvicelPH 101 as a carrier, with different drug concentrations (40%, 50% and 60% *w/w*), showed % cumulative drug release ranging from 67.373% to 88.873% at the 120 min time point.

Formulation batch F-4, F-5, and F-6, which were prepared with Fujicalin as a carrier with different drug concentrations (40%, 50%, and 60% *w/w*), showed % cumulative drug release ranging from 76.85% to 94.61% at a 120 minute time interval.

Formulation batch F-7, F-8, and F-9, which were prepared with Neusilin US2 as a carrier, with different drug concentrations (40 %, 50 %, and 60 % *w/w*), showed a cumulative drug release percentage ranging from 79.617% to 98.033% within a 120-minute time interval. Figure 5 depicts the *in vitro* drug dissolution profile of formulation batches F-7, F-8, and F-9.

Formulation batch F-7 is considered to be an optimized formulation with a maximumcumulative drug release of 98.033% after 120 minutes.

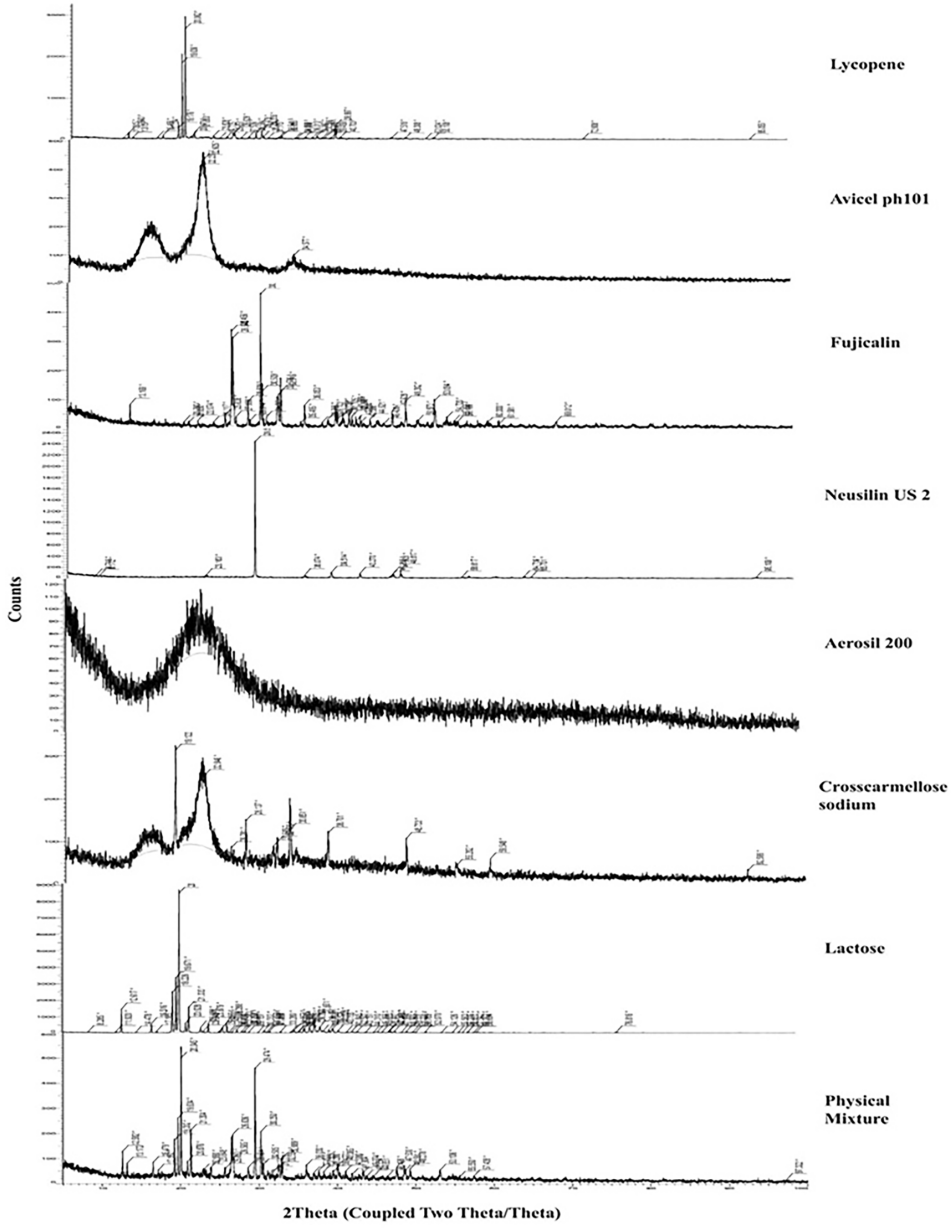

Figure 4. Stacked PXRD of lycopene, excipients, and physical mixture
PXRD: Powder X-ray Diffraction

It was observed that formulation batches prepared with Neusilin US2 as a carrier have higher drug release than those prepared with Fucigeland Avicel PH 101.

The reason behind this phenomenon is that Neusilin US2 (300 m^2/g) has a higher specific surface area than fujicalin (40 m^2/g) and Avicel PH 101 (1.18 m^2/g). A high specific surface area indicates high porosity. The carrier with high porosity enhances the penetration of the dissolution medium, thereby leading to improved dissolution.

It was also observed that the formulation batches with 40% drug concentration showed a higher drug release than formulation batches with 50% and 60% drug concentration.

It has occurred because formulation batches with 40% drug concentration have a higher amount of non-volatile solvent than formulation batches with 50% and 60% drug concentration. The increase in the amount of non-volatile solvent causes improved dissolution due to an increase in drug surface area, enhanced aqueous solubility, and better wetting properties of the drug.

Mathematical modeling

The *in vitro* drug dissolution profile of the F-7 formulation batch was fit to models such as the Zero Order model, Higuchi model, First Order model, Krosmeyer-Peppas model, Hixson-Crowell model, *etc*., to characterize the drug release mechanism. Figure 6 depicts the Higuchi model for F-7. The value of the regression coefficient (R^2) was determined in each model to choose the best-fit model. It was found that the Higuchi model for F-7 has the highest R^2 value of 0.989, and it was selected as the best fit model.

The Higuchi model is used to describe the dissolution profile of the matrix system in which drug diffusion takes place. The dissolution medium enters the formulation, and the drug is released from it by the diffusion process. Thus, optimized formulation F-7 follows a diffusion-typedrug release. In this model, the fraction of drug released is dependent on the square root of time. Such a model is usually followed by matrix systems. It is depicted by the following equation:

$$Q = K_H\sqrt{t}$$

Where,

Q = cumulative amount of drug release at *time t*

K_H = Higuchi dissolution constant

Table 3. Post-compression parameters evaluation of the liquisolid compacts

Batch number	Thickness (mm)	Weight variation (mg)	Hardness (kg/cm²)	Friability, %	Disintegration time (min)	Drug content, %	Cumulative drug release (120 min) %
F-1	4.00±0.105	200.105±2.452	4.166±0.288	0.439±0.276	6.409±0.17	97.036±2.259	88.873±0.095
F-2	3.98±0.138	201.32±4.214	4.5±0.5	0.526±0.034	6.433±0.138	93.467±2.806	75.873±0.989
F-3	4.09±0.190	199.72±4.389	5.5±0.5	0.582±0.157	5.444±0.106	94.982±0.556	67.373±0.667
F-4	3.93±0.075	200.855±3.037	4.33±0.288	0.373±0.301	7.656±0.071	95.487±3.097	94.610±0.144
F-5	4.10±0.057	200.585±2.529	6.166±0.288	0.585±0.089	9.021±0.395	95.218±1.741	86.060±0.629
F-6	4.10±0.057	200.28±2.04	4.666±0.288	0.272±0.333	6.781±0.248	96.127±2.176	76.850±1.439
F-7	3.86±0.075	200.79±2.272	5.166±0.288	0.354±0.166	8.351±0.188	95.252±2.042	98.033±0.748
F-8	3.99±0.080	200.91±2.058	5±0.5	0.493±0.258	6.491±0.33	95.925±2.312	88.983±1.396
F-9	4.07±0.229	200.095±1.907	4.666±0.763	0.44±0.314	6.613±0.152	95.555±1.924	79.167±0.677

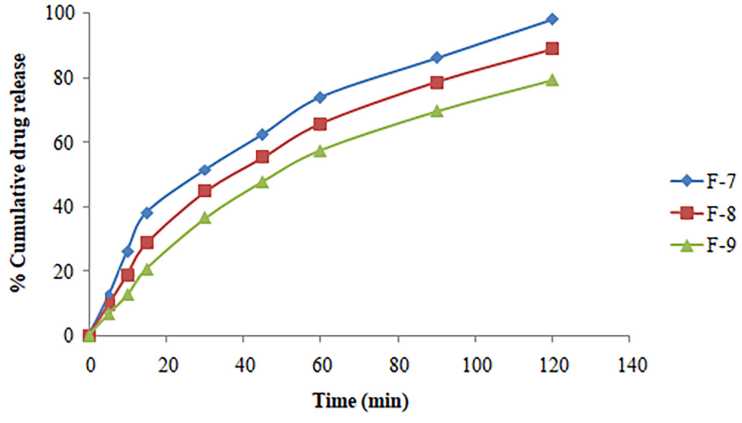

Figure 5. Graphical representation of the *in vitro* drug dissolution profile of formulation batches F-7, F-8, and F-9

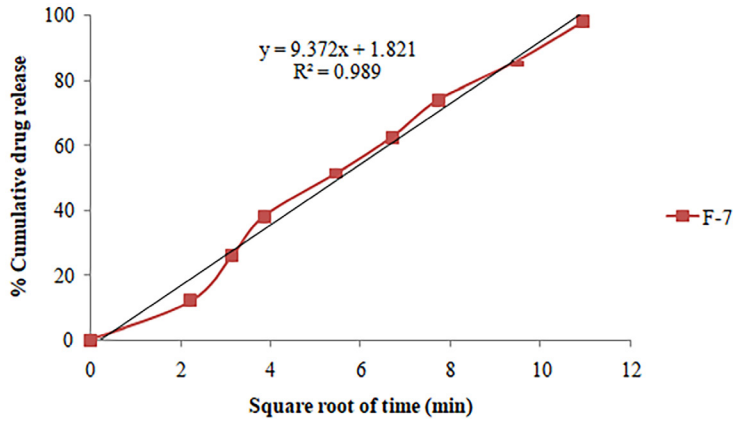

Figure 6. Higuchi model for F-7

DISCUSSION

The IR spectral characteristic peaks were observed for the physical mixtures and the results showed that lycopene showed no evidence of physical or chemical interaction. All FTIR spectra suggest that the drug and excipients had no interaction. In glycerin, lycopene has shown the maximum solubility of 152.35 mg/mL, this would facilitate the dissolution rate by improving the surface area as it will be molecularly dispersed.

Neusilin (Magnesium Alumino-metasilicate) has a high SSA and liquid absorption capacity, which aids in the assimilation of a greater amount of liquid material into the liquisolid structure. *In vitro* drug release study demonstrates that lower drug proportion in glycerin results in higher drug release, on the other side, the Higuchi model indicates that drug release is dissolution rate controlled, as evidently shown in themathematical modelling.

CONCLUSION

This research aimed at developing lycopene-based liquisolid compacts with improved dissolution. Different formulation batches of lycopene-based liquisolid compacts were formulated using varying carrier and drug concentration percentages. The results indicated that IR spectroscopy and PXRD showed no drug-excipient interaction. Moreover, all formulation batches passed pre-compression and post-compression evaluation. F-7 formulation batch with Neusilin as carrier and 40% drug concentration having 98% *in vitro* release was found to be the optimized formulation with maximum dissolution. Thus, this research concludes that Neusilin isthe most effective carriercompared toFujicalin and Avicel. Thus, this technique that uses Neusilin as a carrier can toenhance the dissolution of lycopene.

Ethics

Ethics Committee Approval: Not required.

Informed Consent: Not required.

Footnotes

Authorship Contributions

Concept: J.S., Design: J.S., Data Collection or Processing: S.S., Analysis or Interpretation: S.S., J.S., Literature Search: S.S., Writing: S.S.

Conflict of Interest: The authors declare no conflicts of interest.

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ORIGINAL ARTICLE

Nanobubble-Enhanced Oral Delivery of Bortezomib: Optimizing Preparation and Characterization through Design of Experiment (DOE)

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ABSTRACT

Objectives: Bortezomib (BTZ) functions as an androgen receptor signalling inhibitor, is used for the treatment of prostate cancer, and has been sanctioned by the United States Food and Drug Administration. The medicinal applications of BTZ are impeded by low solubility, first-pass metabolism, and restricted bioavailability. This study aimed to develop and enhance polylactic acid-co-glycolic acid (PLGA) nanobubbles (NBs) as a sustained-release mechanism for BTZ, thereby augmenting stability and bioavailability.

Materials and Methods: Seventeen experimental runs were conducted to optimize drug-PLGA NBs using a three-factor, three-level Box-Behnken Design. The improved formulation comprised 30 mg of medication, 250 mg of PLGA, and 2.0% *w/v* polyvinyl alcohol as a stabilizing agent.

Results: The NBs exhibited a particle size of 186.9±13.9 nm, a polydispersity index of 0.146±0.042, and a zeta potential of -21.4±2.28 mV, along with an entrapment efficiency of 66.12±1.48%. Fourier transform infrared spectroscopy, differential scanning calorimetry, and X-ray diffraction analysis verified the absence of drug-polymer interactions, whereas scanning electron microscopy demonstrated uniform spherical nanoparticles. *In vitro* experiments demonstrated superior drug release, and stability assessments indicated no major alterations after one month. Pharmacokinetic studies in rats demonstrated an elevated C_{max} (1.69) and area under the curve from time 0 to t (1.63), signifying enhanced sustained release and absorption. The results underscore the capability of BTZ-loaded PLGA NBs to augment drug kinetics and bioavailability, hence facilitating targeted distribution and enhanced therapeutic efficacy.

Conclusion: This investigation offered significant insights into the factors influencing oral absorption in NB formulations, which can guide future methods for oral medication development. BTZ-loaded PLGA nanobubbles showed promising results by enhancing oral absorption and improving pharmacokinetics in the study, which points to their potential use in sustained-release drug delivery. These findings offer a stepping stone toward nanomedicine via the oral route in future drug development.

Keywords: Acoustics, Box-Behnken design, BTZ, design of experiment, nanobubbles, PLGA

INTRODUCTION

B lymphocytes are a type of white blood cell that is impacted by multiple myeloma (MM), a cancerous condition. It is identified by the aberrant proliferation of a single bone marrow-derived plasma cell that produces monoclonal immunoglobulins.[1] MM is the second most common haematological malignancy, accounting for 1% of all cancers and 13% of haematological cancers.[2,3] One promising approach for treating MM is targeting the ubiquitin proteasome system (UPS).[4] The UPS is responsible for degrading defective proteins by recognizing poly-ubiquitin chains attached to them. Inhibiting proteasome activity leads to the accumulation of cells, triggering apoptosis or programmed cell death. Proteasome inhibitors have shown

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potential as antitumor agents and can also inhibit angiogenesis (anti-angiogenic properties) by blocking the UPS. Bortezomib (BTZ) (Velcade®), an approved drug by the Food and Drug Administration, is commonly used as a first-line treatment for MM and mantle cell lymphoma.[5] By acting as a proteasome inhibitor, BTZ binds reversibly to the chymotrypsin-like subunit of the 26S proteasome. This mechanism inhibits the proteasome, ultimately halting the breakdown of various pro-apoptotic factors.[6] BTZ is offered in intravenous (IV) and subcutaneous formulations. Mannitol, at a concentration of 10 mg per gram of BTZ, is used to improve solubility upon reconstitution (Velcade®). The sugar molecule helps in BTZ dissolution by forming a BTZ trimer and boronate ester, through covalent binding with the boronic acid moiety of BTZ. This ester prevents trimer formation, which could reduce water solubility. Moreover, mannitol, a highly water-soluble polyol, lowers the pKa of BTZ by three units, further increasing water solubility.[7]

The main issue with the current formulation of BTZ, which utilizes mannitol for solubility enhancement, lies in its lack of suitability for long-term drug delivery. Mannitol aids in BTZ dissolution and increases water solubility. However, its use as a cosolvent in the formulation may pose challenges for sustained drug release over an extended period. Mannitol's rapid dissolution and clearance from the body may lead to a shorter duration of therapeutic effect, necessitating frequent administration to maintain therapeutic levels of BTZ. Therefore, alternative formulations or delivery strategies that ensure sustained release and prolonged therapeutic efficacy may be required for long-term use of BTZ.

Despite the favourable clinical results, the use of BTZ has been limited because it depends heavily on proteasome function, leading to toxicity. Moreover, the medication is linked to various adverse reactions.[5,8] Additionally, BTZ is faced with pharmaceutical challenges due to its low water solubility (pKa value of 13) and a brief half-life in the bloodstream.[5] The combination of toxicity and unfavourable pharmaceutical characteristics creates substantial barriers to its use in clinical settings. As a result, there is an urgent need for a specialized formulation of BTZ. The various physicochemical and pharmacokinetic properties of BTZ are listed in Supplementary Table 1.

Therefore, a strategy that reduces the pharmacokinetic variations between the fed and fasting states must be developed. Strategies such as liposomal formulations, polymeric particles, and self-microemulsifying drug delivery systems are designed to improve the bioavailability of the drug.[9-11] *In vivo* performance, scalability issues, and the need for costly and specialised branded excipients are the obstacles with the reported approaches. Beyond enhancing solubility, an approach that offers the potential to target drug molecules specifically to diseased tissues while reducing their concentration in normal tissues is essential. Such a targeted delivery system will improve stability in bodily fluids, optimize concentration and release kinetics in the bloodstream, and enhance pharmacokinetic and pharmacodynamic properties. All these factors collectively contribute to heightened efficacy and reduced side effects. One example is the Smart delivery system, which is currently focused on cancer treatment.

At the forefront of these innovative delivery systems are smart delivery systems, which have garnered significant attention and focus, particularly in the realm of cancer treatment. By leveraging advanced technologies and principles, delivery systems that are precisely targeted and regulated represent a state-of-the-art method for administering medication aimed at navigating drugs to their intended targets within the body. Through the integration of sophisticated mechanisms, these systems hold the potential to revolutionize cancer therapy by maximizing efficacy and minimizing the impact on healthy tissues.

A smart drug delivery system possesses the ability to react to sudden changes in its surroundings, particularly those brought on by chemical stimuli. Extensive research has been conducted on the application of pressure waves and ultrasonic (US) triggers in drug delivery systems, which are dependent on external stimuli.[12]

The potential for targeted therapy in a range of medical applications has been enhanced by the broad exploration of these external stimuli to establish precise control over medication release.

Nanobubbles (NBs), or microscopic bubbles at the nanoscale, are used in numerous fields, most notably in drug delivery systems. Their amazing stability, high internal pressure, and large surface-to-volume ratio are only a few of their outstanding physical characteristics.[13]

Drugs and other therapeutic agents can be efficiently delivered to cancer cells by using NBs as carriers. NBs can vary in size from 1 nm to 500 nm, and their dimensions can be precisely tailored to the nanoscale using amphiphilic polymers such as Pluronic or surfactants. By improving blood-brain barrier penetration, NBs provide opportunities for focused drug delivery in neurological diseases, including Alzheimer's and Parkinson's. They also show potential in cardiovascular illnesses for precise thrombolytic therapy, enhancing clot breakup with fewer side effects. Research has been done on both polymeric and lipidic NBs; however, the instability of lipid-based NBs leads to breakdown and a shorter circulation period. Poly (lactic-co-glycolic acid), or PLGA, is a special kind of nano/micro biomaterial that can be used for diverse applications such as targeted medication delivery, molecular diagnostics, tissue engineering, and gene transfer.[14] Its high stability, biodegradability, and ease of chemical modification are the favourable characteristics of the PLGA.[15]

To the best of our knowledge and based on available literature, there are no prior studies documenting the utilization of PLGA NBs for delivering BTZ to cancer cells. This study aimed to develop and enhance polylactic acid-co-glycolic acid (PLGA) NBs as a sustained-release mechanism for BTZ, thereby augmenting stability and bioavailability. The research involves the preparation of BTZ-containing NBs using PLGA, followed by comprehensive *in vitro* characterization, including analysis

of particle size (PS), size distribution, zeta potential (ZP), morphology, release kinetics, and subsequent *in vivo* evaluation.

MATERIALS AND METHODS

The pure drug BTZ was acquired from Dr. Reddy's Ltd., Hyderabad, India. Sigma Aldrich, USA, supplied Poly (D, L-lactide-co-glycolide) 50:50 with an intrinsic viscosity of 0.22 dL/g and a molecular weight of 25,000. Polyvinyl alcohol (PVA; Mw 30,000-70,000) was purchased from Sigma Aldrich (St. Louis, MO, USA). Borviz 2 injection was purchased from Intas Pharmaceuticals Ltd., Ahmedabad, India. Antipyrine was procured as a sample from M/s Yarrow Chemicals Pvt., Ltd., Hyderabad. Isopropanol and dichloromethane (DCM) were acquired from S.D. Fine Chemicals, Hyderabad. We purchased acetonitrile from Qualigens, India. The cells were purchased from NCS Pune, Maharashtra, India. All the media components were purchased from Gibco, USA, and Invitrogen, USA.

Analytical method development using reverse-phase high-performance liquid chromatography

Chromatographic analysis of BTZ was executed using a Shimadzu Prominence model LC-20 AD, equipped with an ultraviolet detector set at 230 nm. A reverse phase Luna C-18 column (150 mm × 4.6 mm *i.d.*, 5.0 µm PS and 100 Å pore size) maintained at 40.0±0.1 ˚C was employed. The mobile phase is composed of a mixture of acetonitrile and ammonium phosphate (buffer pH 4 adjusted with acetic acid) (60:40 *v/v*). The mixture was sonicated for 45 minutes for degassing purposes and then filtered through a 0.45 µm pore diameter Whatman filter paper. The flow rate was adjusted to 1.0 mL/min. A standard stock solution of the drug at a concentration of 1000 µg/mL was prepared, followed by the creation of various working standard solutions ranging from 0.5 to 100 µg/mL through serial dilutions. A stock solution (1000 µg/mL) of Antipyrine as IS (internal standard), after appropriate dilution, was included in the experiment. Retention time of drug and internal standard was 4 minutes and 6.8 minutes, respectively.[10]

BTZ-NBs formulation development and optimization

BTZ-loaded PLGA NBs were synthesized using solvent evaporation with ultrasound assistance following a reported method with slight modifications. Initially, a homogeneous solution was formed by dissolving PLGA (200 mg) in a water-immiscible solvent, DCM. The drug BTZ was then added to create a dispersion, which underwent sonication for five minutes at 45% amplitude, in an ice bath using a Digital Sonifier S-250D (Branson US, Danbury, USA). Next, the drug dispersion was combined with 25 mL of chilled 2.0% PVA solution and homogenized at 13222 rpm for 10 minutes using a high-speed homogenizer. The formulation was then subjected to sonication using a US probe at 30 W for 3 minutes. A 2.5% *v/v* isopropanol solution (25 mL) was added to the emulsion and mechanically stirred for 5 hours to remove the DCM. Subsequently, the resultant product was centrifuged at 8000 rpm for five minutes. The product was washed with distilled water after discarding the supernatant. This centrifugation and washing process was repeated three times. The NBs were freeze-dried for 36 h without exposure to light using a LYPH LOCK 4.5 (Labconco Corporation, Kansas City). Later, C3F8 gas (perfluoropropane) was added to the lyophilization chamber through a vial connector at a flow level of 50 mL/min for 1 minute. After this, the screw vials were tightly sealed for further analysis.[16] BTZ-loaded PLGA NB optimization was achieved by implementing a three-factor, three-level BBD. A total of 17 experimental runs were conducted, including three replicated centre points.[17]

The three independent variables were the amount of PVA *(w/v)*, homogenization speed (rpm), and homogenization time, each at three levels of variation: low (-1), middle (0), and high (1). The response (three dependent variables) were PS (Y1), polydispersity index (PdI) (Y2), and encapsulation efficiency (% EE) (Y3); Table 1 lists their respective ranges. Utilizing response surface charts and contour (2D) plots, the response surface search was carried out with Design Expert® (Version 12.0.3.0, Stat-Ease Inc., Minneapolis, MN).

Characterization and evaluation

Measurements of PS, PdI, and ZP

A Zetasizer (Malvern Instruments, UK) was used with DLS theory (dynamic light scattering) to calculate the PS, PdI, and ZP of BTZ NBs after tenfold dilution of the sample with double-distilled water.[18]

Percentage entrapment efficiency (% EE)

The % EE of the drug in the NBs can influence the therapeutic efficacy, stability, and release kinetics of the loaded compounds within the NBs. DCM was used to dissolve a particular quantity of NBs containing the loaded drug (BTZ) NBs. The complex was dissolved by subjecting the solution to sonication for 10 minutes. The resultant solution was then suitably diluted and assessed using the above-mentioned HPLC method.

The following equation was used to calculate the same:

$$\text{Entrapment efficiency \%} = \frac{\text{(total amount of the drug-free drug)}}{\text{(amount of drug)}} \times 100$$

Table 1. Factors influencing the experiment's design

		Levels		
	Independent variables	**Low (-1)**	**Medium (0)**	**High (+1)**
A	Amount of PVA (% *w/v*)	1.0	1.5	2.0
B	Homogenization speed (rpm)	10000	12500	15000
C	Homogenization time (mins)	5	10	15
	Dependent variables	**Restrictions**		
Y1	Particle size (nm)	Minimize		
Y2	Polydispersity index	Minimize		
Y3	EE (%)	Maximum		

PVA: Polyvinyl alcohol, EE: Encapsulation efficiency

Morphology using scanning electron microscopy (SEM)

Using a Quanta FESEM 250, the structure of the NBs and the drug was captured. Before testing, the sample was mounted on an aluminium pin stub after being mounted with double-sided carbon tape and Au-sputter coated utilizing an ion sputter. The specimen was then analysed at an operating distance of 10 millimetres, with an acceleration current of 30 kV and a magnification of 500-10,000 times.[19]

Fourier-transform infrared (FTIR) spectroscopy

The spectrum of FTIR was obtained using a Perkin-Elmer spectrometer (Model 1600; USA). The pure drug, PLGA, physical mixture (PM), and the optimized drug-loaded NBs were all analysed at wave numbers 4000-450 cm-1 with a resolution of 1.0 cm-1.[20]

Differential scanning calorimetric study and X-ray diffraction pattern (XRD)

DSC (DSC-60, Shimadzu Corp., Japan) was employed to ascertain the drug's physical structure and the potential for chemical interactions with the excipients. Samples of 3-5 mg (pure drug, blank NBs, PLGA, optimized drug-loaded NBs, and NBs stored for three months) were subjected to heating (50-400 °C, 5 °C/min) in folded aluminum pans in a nitrogen environment before being subjected to DSC analysis, following calibration using Indium and Lead standards. The melting point (MP) and the enthalpy of fusion were computed automatically. The X-ray diffraction patterns of the pure drug, the PM, and the optimized formulations were obtained using a Philips X-ray diffractometer (PW-1710) operated with a graphite monochromator and Ni-filtered Cu Kα radiation (at 100 mV and 40 kV). The samples were scanned between 2° and 60° 2 theta (θ) angle with an average step size of 0.045° and a duration per step of 0.5 seconds.[21]

Drug release

The release studies of pure drug and optimized drug-loaded PLGA NBs were carried out using *in vitro* experiments with a shake flask equipped with a dialysis bag. Following encapsulation in dialysis membranes, the specimens were transferred into a conical flask containing phosphate buffer (pH 7.4), maintained at 37 °C, and subjected to constant rotation at 100 rpm. One mL of sample was removed from the outer solution and replaced with brand-new PBS at pH 7.4 at predefined intervals. These aliquots were filtered and analysed using the HPLC method at 230 nm to measure drug release. Three duplicates of the experiment were carried out.[22]

Stability studies

The optimized formulation's stability was evaluated by storing it at three temperatures (4 °C, 25 °C, and 40 °C) at 75% relative humidity. Regular intervals were set to measure PS, PdI, and EE% % changes in the samples.[23]

Pharmacokinetic studies (PKs)

Male Wistar rats with an approximate weight of 200±20 g and an age of 4-5 weeks were procured from the National Institute of Nutrition situated in Telangana, India. The animal protocol was designed and approved by the Institutional Animal Ethics Committee of Teegala Ram Reddy College of Pharmacy (approval number: 1447/PO/Re/S/11/CPCSEA-97/A, dated: 17.02.2024). For about a week, animals were exposed to natural light/dark settings, acclimating to a relative humidity of 40–60% and a temperature of 20 °C±2 °C. Then they were split into three groups of six animals randomly. An optimized drug-loaded NB (0.2 mg/kg BW) and the pure drug (dispersed in 0.5% *w/v* carboxymethyl cellulose) were administered by the oral route, while the reference (marketed product) was given by the IV route. The animal blood was obtained from the retroorbital plexus (300 µL) and then transferred into sterile test tubes with EDTA at specific intervals (0.25, 0.5, 1, 2, 4, 6, 12, 24, 48, and 72 h). Blood samples were centrifuged at 7500 rpm for ten minutes using an Eppendorf centrifuge. The resulting plasma was further analysed using HPLC.[10]

Using the protein precipitation method, the drug was recovered from plasma samples. By adding 250 µL of acetonitrile to 50 µL of rat plasma and vortexing the mixture, the drug was effectively extracted from the plasma. After centrifuging the supernatant for 12 minutes at 8000 rpm, chromatography was used to analyze it at a wavelength of 230 nm. Non-compartmental analysis using WinNonlin (version 3.1; Pharsight Corporation, USA) was employed to calculate the C_{max} (maximum plasma concentration), AUC_0-72 (area under the plasma concentration vs. time curve from 0 to 72 h), T_{max} (time to reach the maximum plasma concentration), Kel (elimination rate constant), and t1/2 (half-life).

Statistical analysis

Non-compartmental analysis WinNonlin (version 3.1; Pharsight Corporation, USA) was employed to calculate all pharmacokinetic parameters. All the data were expressed as mean ± standard deviation.

RESULTS

BTZ-NBs formulation development and optimization

The study aimed to formulate BTZ NBs by integrating ultrasound technology with solvent evaporation. The drug had been mixed with PLGA that had been dissolved in DCM, sonicated, and added to a cooled 2.0% *w/v* PVA solution. After that, a 3-minute sonication at 30 W and high-speed homogenization were performed on the sample. Isopropanol solution (2.5% *v/v*) was used to extract DCM.[24,25]

BTZ PLGA NBs formulation optimization by Quality by Design emphasizing preciselyby monitoring critical quality attributes (CQAs) to achieve and maintain QTPP was carried out.[26] The current study selected PS, PdI, and EE as CQAs (Table 2). Multiple linear regression analysis (2FI) constructed polynomial models (quadratic, two-factor, and linear). The model selection used R^2, predicted R^2, adjusted R^2, and coefficient of variance (C.V.). Analysis of variance (ANOVA) assessed the impact of variables on responses.

Table 2. Box–Behnken Design and the experimental data

Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
A: Stabilizer concentration, %	B: Homogenization speed, rpm	C: Homogenization time, mins	PS (Y1), nm	PdI (Y2)	EE % (Y3)
1.5	15000	5	207.12	0.332	52.26
1	12500	5	302.88	0.416	70.03
2	12500	15	234.4	0.229	78.16
2	12500	5	244.12	0.262	61.9
1	12500	15	296	0.488	73.25
1.5	10000	5	302.03	0.32	71.94
2	10000	10	197.89	0.229	65.16
1.5	12500	10	182.78	0.182	66.44
2	15000	10	199.64	0.132	64.04
1.5	12500	10	209.26	0.234	66
1.5	12500	10	209.1	0.229	64.77
1.5	12500	10	204.7	0.244	64.08
1.5	15000	15	258.44	0.288	71.41
1	10000	10	256.3	0.418	76.47
1.5	12500	10	210.5	0.183	66.1
1.5	10000	15	199.2	0.36	68.36
1	15000	10	254	0.336	56.66

PS: Particle size, PdI: Polydispersity index, EE: Encapsulation efficiency

PS

A small size of NBs resulted in a significantly higher surface area-to-volume ratio, enhancing their stability, penetrability, and reactivity in targeted drug delivery 17. After 17 trials, PS ranged from 182.78 to 302.88 nm. The model's *f* value of 21.43, with a 0.03 percent chance of being noise, confirmed its "quadratic" nature and that the lack of fit is insignificant. The various perturbation plots of PS, PdI, and % EE are shown in Supplementary Figure 1. ANOVA found variables with p-values below 0.0500 that significantly impacted the response. The "lack of fit *f* value" (0.77) implied that any lack of fit was not statistically significant. There was a 56.84% chance that a "Lack of Fit F-value" of this extent would occur due to random noise, undermining the model's reliability. The contour plots (CP) and 3D response surface plots (RSP), illustrating variable influences on PS, were depicted in Figure 1. The R2, corrected R2, and anticipated R2, with values of 0.9650, 0.9200, and 0.7604, respectively, showed a model precision of 13.19, surpassing the required value of 4.

Stabilizer concentration (A), with a high negative coefficient, was the most influential factor affecting PS, as it demonstrated that alterations in this factor resulted in a corresponding change in PS. Stabilizer concentration, homogenization speed, and time, denoted by variables A, B, and C, also played a role in PS, although to a lesser extent than the other factors.

The interaction terms (BC) suggested a combined effect of these variables, and this had a significant impact on PS. the positive coefficients for quadratic terms like A2 and C2 indicate that the squared values of A and C have a nonlinear relationship with PS, potentially leading to changes in PS that are not directly proportional to changes in the variables. Model terms (A, B, C, BC, A^2, and C^2) had *p* values <0.0500, signifying their effect. The resulting regression equation was:

$PS = 217.63 - 28.79\,A - 23.80\,B - 24.46C + 1.01\,AB - 0.3550\,AC + 19.27B\,C + 25.67\,A^2) - 1.98\,AB^2 + 10.10\,C^2$

PdI

The PdI is a dimensionless metric that quantifies the broadness of the PS distribution.[17]

Typically, it falls between 0 and 1. Formulations exhibited PdIs from 0.132 to 0.488, with a model *f* value of 19.68, which indicated a significance of the proposed "quadratic" model and an insignificant lack of fit (*f* value 0.78). There was a 56.38% chance that a "Lack of Fit *f* value" of this magnitude would arise due to random noise, underscoring the model's reliability.

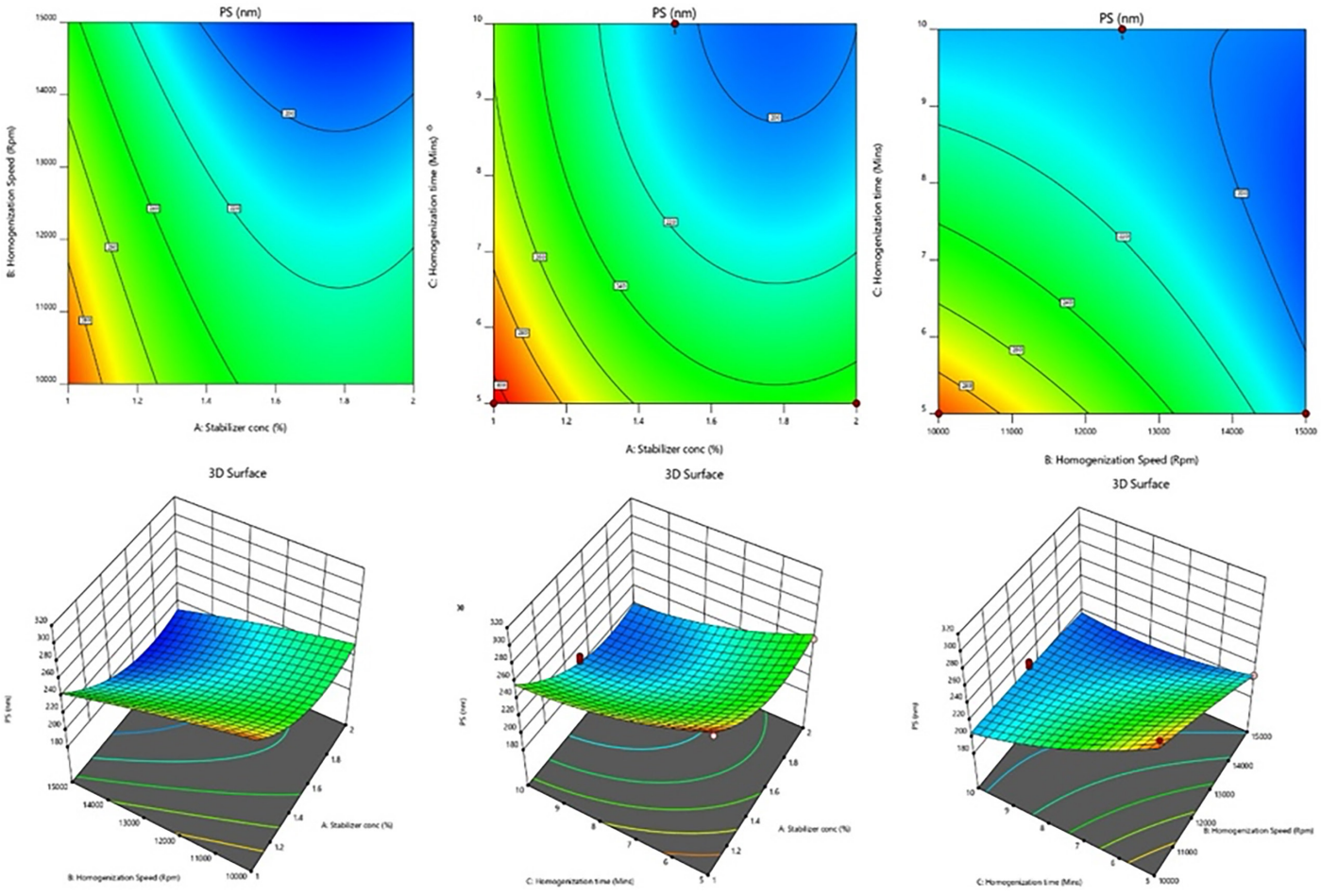

Figure 1. CPs and RSPs illustrating variable effects on PS
CPs: Contour plots, RSPs: Response surface plots, PS: Particle size

Regression coefficients (R2, adjusted R2, and anticipated R2) were 0.9620, 0.9131, and 0.7383, which showed the model's usefulness with precision exceeding the necessary value (15.5323). Model terms (A, C, A2, and C2) had *p* values <0.050, signifying a substantial impact. The resulting regression equation is:

$$PDI = 0.2348 - 0.0876\ A + 0.0194\ B - 0.0430\ C - 0.0037\ AB + 0.0131\ AC - 0.0105\ BC + 0.0440\ A^2 + 0.0203\ B^2 + 0.0226\ C^2$$

Positive coefficients signify a rise in the associated variable(s), which increases PdI, while negative coefficients indicate a decline, which reduces PdI.[27] An increase in variables A and C led to a decrease in PdI, whereas higher values of B (homogenization speed) led to higher PdI. All quadratic terms have positive coefficients, indicating that increases in the squared values of variables A, B, and C lead to increases in the PdI. The response surface and contour plots illustrating variable effects on the PdI are shown in Figure 2.

Percentage entrapment efficiency (% EE)

Impact on % EE ranged from 52.26% to 78.16%. The NB with high entrapment is always desirable, as it helps reduce the dose of the drug.[27] The model's *f* value, 63.30, with a 0.01% chance, is likely due to noise, indicating significant and negligible fit error for the suggested "quadratic" model. The F-value for lack of fit (1.41) was statistically insignificant based on pure error, with a 36.19% probability of being noise. ANOVA identified significant factors (*p* value <0.1000), which led to the removal of non-significant variables. The consequence of variables on ZP, as demonstrated by CP and RSP, is shown in Figure 3. The regression coefficients, R2, adjusted R2, and anticipated R2 were 0.9879, 0.9723, and 0.8908, respectively. The predicted R2 aligns closely with the adequate R2, differing by 0.2. The model, evidenced by an adequate precision (signal-to-noise ratio) of 31.5008, surpassing the necessary value of 4, proved helpful in exploring the design space. Figure 4 illustrates a CP and 3D SP, which showcase the influence of selected variables on % EE. An elevation in factors A and B, both related to stabilizer concentration, typically results in decreased entrapment efficiency. In contrast, the interaction terms and quadratic terms increased the percentage of EE. By comprehending the impact of these factors on entrapment, researchers can fine-tune formulations to attain desired levels of efficiency, thereby guaranteeing the effective delivery of substances in

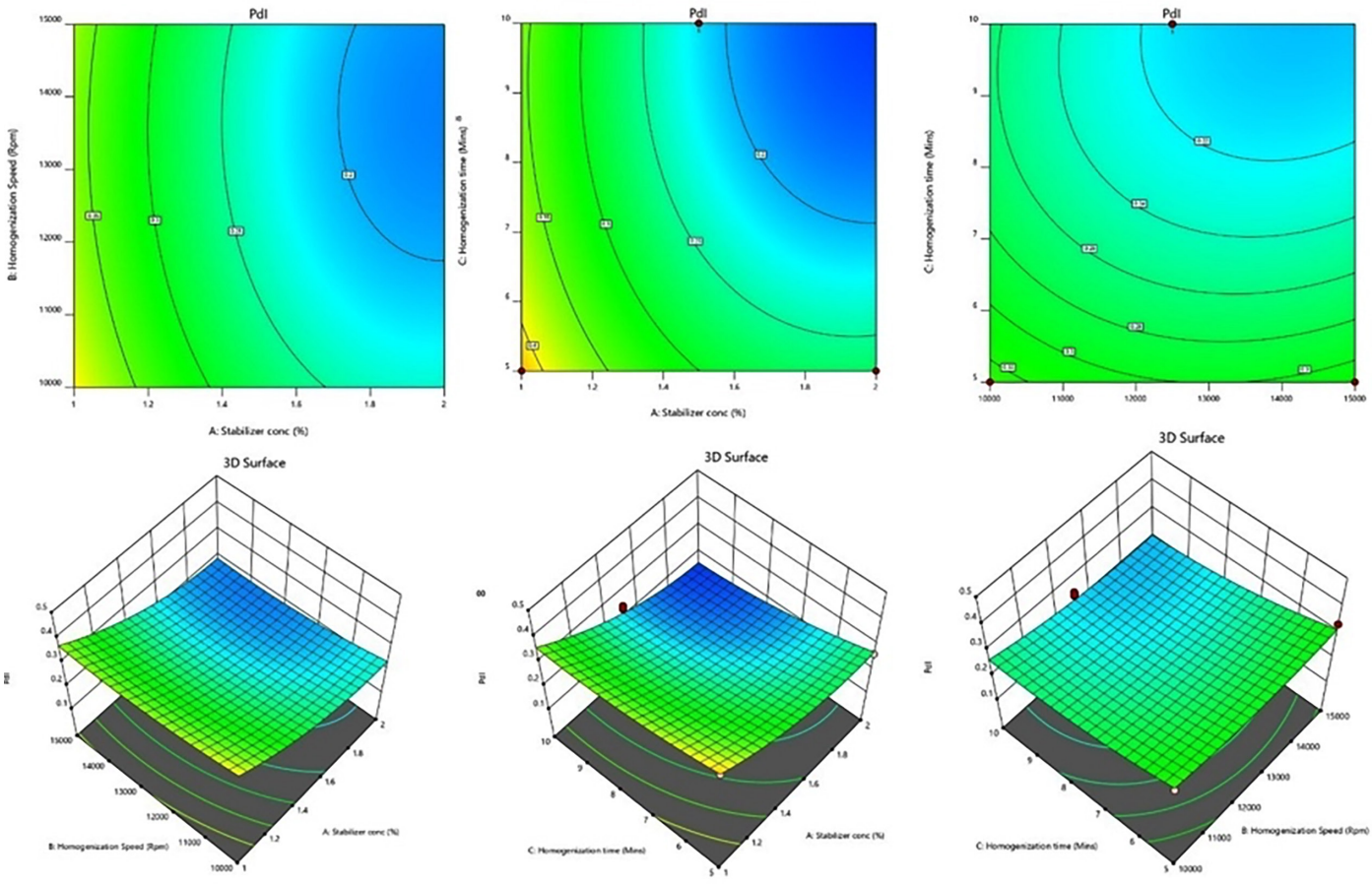

Figure 2. Graphical depiction of contour plots and response surface plots illustrating variable effects on PdI
PdI: Polydispersity index

pharmaceutical or other applications. Model terms (A, B, AB, AC, BC, A^2, B^2, and C^2) had *p* values <0.0500, signifying a significant effect. The resulting regression equation is:

$$EE\% = +64.01 - 2.51\,A - 7.54\,B + 0.7489\,C + 4.67\,AB + 1.63\,AC + 2.84\,BC + 2.47\,A^2 - 2.37\,B^2 + 0.7209\,C^2$$

Characterization and evaluation of NBs

PS, PdI, ZP, % EE, and morphology

PSs and uniformity in the formulation remained consistent, with PS of 186.9±13.9 nm and PdI of 0.146±0.042. A polydispersity value below 0.3 indicated homogeneity. The optimized formulation's ZP, indicative of colloidal particle surface properties, was -21.4±2.28 mV, with a % EE of 66.12±1.48. The PS and ZP of the optimized NBs are shown in Figure 5. NB stability heavily relies on elevated ZPs, which play a crucial role in maintaining electrostatic repulsion among particles, thus preventing their aggregation. This stability is of utmost importance as it ensures the integrity of NBs during storage and administration, ultimately enhancing their performance in drug delivery and medical imaging applications. PS and ZP were shown in Supplementary Figure 2. It was evident from the figure that the drug had a variety of PSs and a non-uniform cubic shape containing particles as small as micrometres. However, after the formation of the NB, the micronized drug particles consistently changed to spherical particles in the nanosize range.

FTIR

Figure 5 illustrates the determination of component compatibility observed for nano formulation, excipients, and plain drug infrared spectra, using a scanning range of 450 cm-1 to 4000 cm-1. Distinctive peaks in the plain medication were observed at 3446.9; 3294; 2937.68; 1747.57; 1663; 1523; 1460; 1394; 1261.49; 1193.98; 1085.96; 1022; 885.36; and 719 cm-1. There was a physical interaction between the drug and stabilizer, as evident in the stability of the apparent peaks and the absence of new peaks in both the PM and the freeze-dried formulation. The FTIR spectral data with wave number and the corresponding groups is given in Supplementary Table 2.

The stability of the evident peaks and the absence of new ones in the physical mixing and freeze-dried formulation indicate that there is physical interaction between the drug and stabilizer.[28]

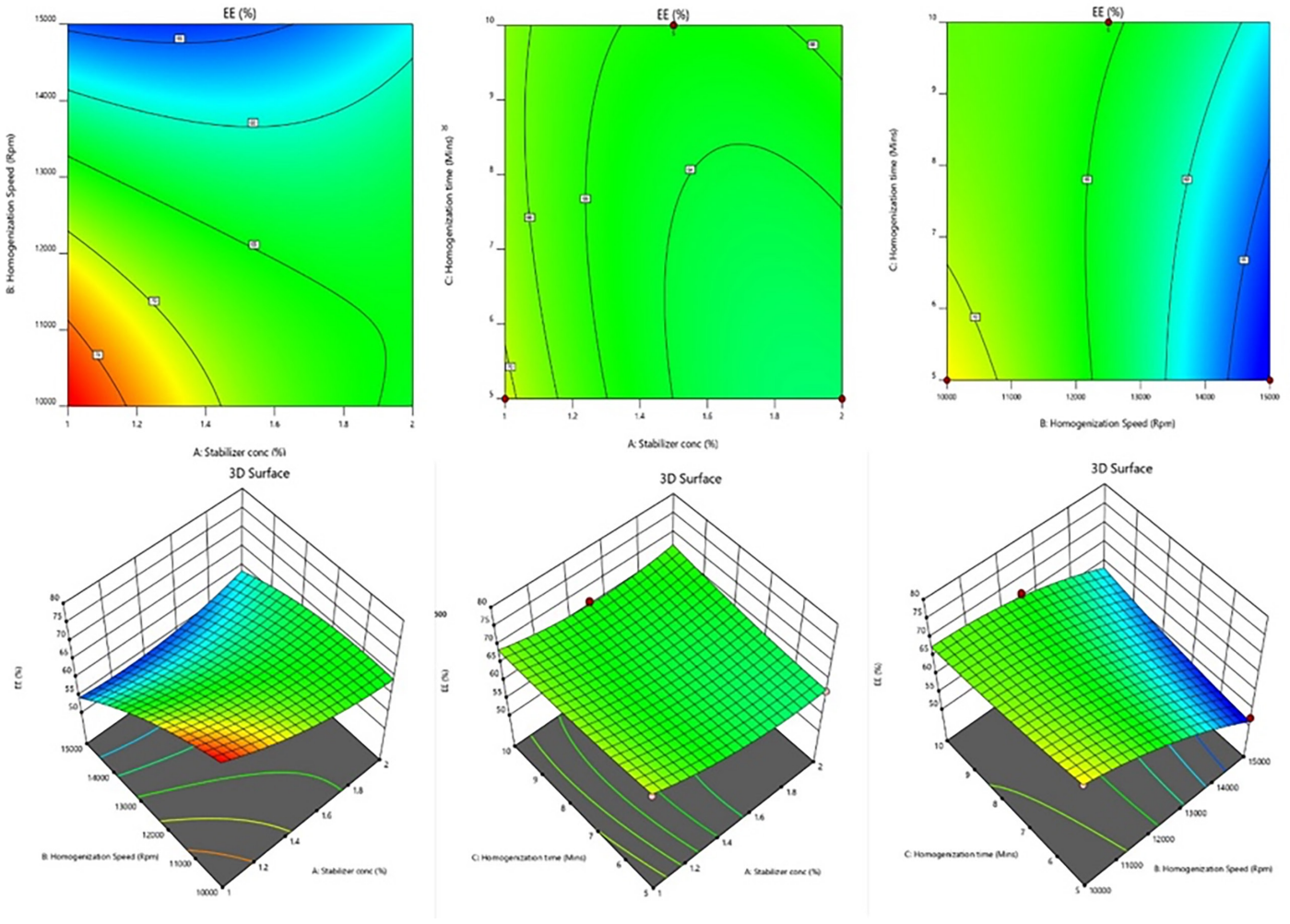

Figure 3. Graphical representation of RSPs and CPs demonstrating its consequences of variables on % EE
RSPs: Response surface plots, CP: Contour plots, EE: Encapsulation efficiency

DSC and XRD

DSC assessment was accomplished to evaluate the thermal characteristics of the drug, PLGA, and the NBs pre- and post-storage for 3 months (Figure 6). The pure drug exhibited a clear endothermic peak at 167.36 ˚C, signifying its MP a broad peak at 287.64 ˚C, and at 43.31 ˚C, indicating its crystalline characteristics.[28]

The thermograph of PLGA exhibited peaks at 61.8 ˚C and 163.17 ˚C. In the thermographs of the NBs, two peaks emerged at 200.52 ˚C and 26.18 ˚C, indicating the minor shifts in the MP of the drug and its confinement within the polymeric structure due to weak intermolecular interactions between the drug and the polymer.

The XRD patterns are depicted in Figure 6B. The drug has displayed firm diffraction peaks (2θ scattered angles of 12.88, 16.52, 18.07, 19.65, 20.52, 21.3, 24.06, 24.2, 24.53, and 33.530) confirming its crystalline nature.[28,29] Previous studies have also reported similar diffraction peaks for the drug under study. However, in the NBs, the characteristic diffraction peaks of BTZ vanished, suggesting the pure drug may have formed a solid-state complex at a molecular level.

Drug release

Figure 7 depicts dissolution profiles of plain drug and drug-loaded NBs with and without acoustic assistance. Drug release from NBs was significantly higher than from a simple drug suspension. Notably, ultrasound assistance increased drug release. The cumulative drug release at 8 hours was 17.24±3.32%, 40.04±4.61%, and 76.84±4.04% for plain drug, NBs without and with acoustic activation, respectively. By 48 h, over 98.14±8.47% was released from NBs with acoustic assistance and 68.02±8.26% without acoustic aid, but in the case of the pure drug, only 33.1±4.60% of the drug was released. Drug release occurred due to cavitation collapse induced by acoustic waves and disrupted NB structures, enabling rapid medication release. Acoustic waves, characterized by meticulous control and a non-invasive nature, offer accurate drug delivery and targeting abilities. These findings confirmed that ultrasound assistance plays a pivotal role in enhancing drug release from the NBs, potentially through the cavitation effect induced by ultrasound. Ultrasound stability studies indicated the transformation of the gas core, where nanodroplets turn into bubbles, known as acoustic droplet generation. Under the influence of ultrasound,

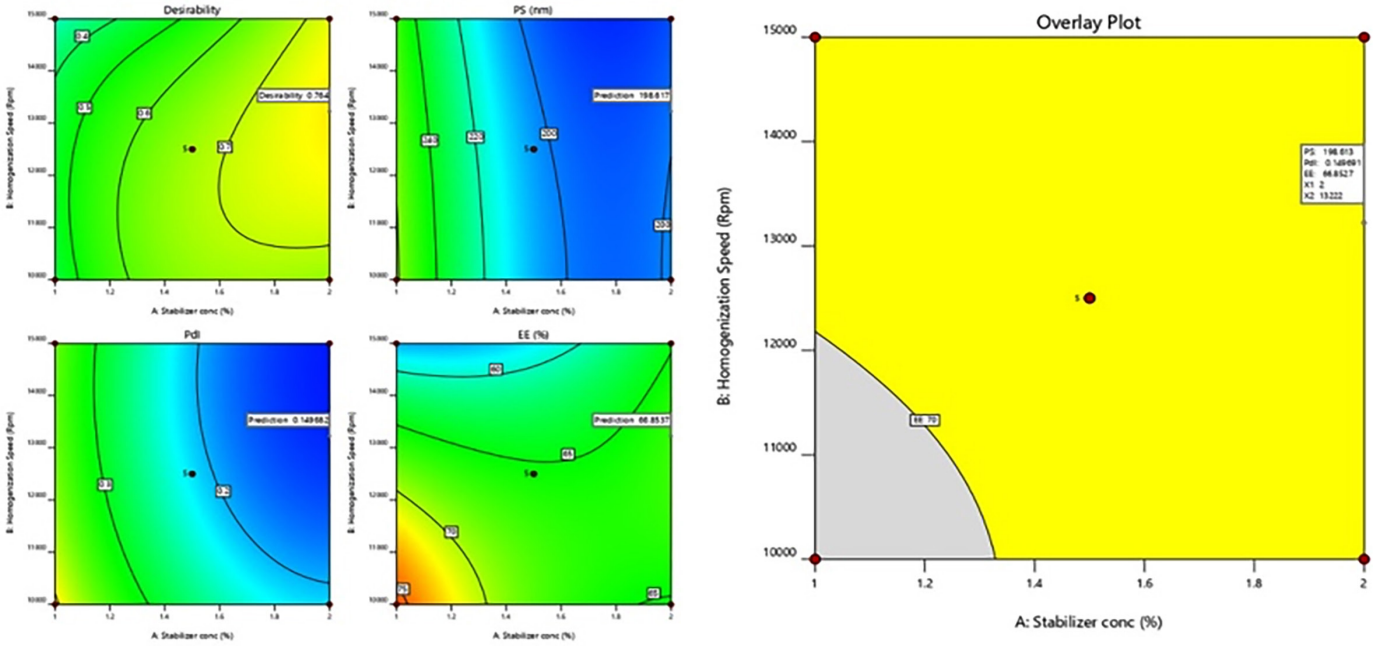

Figure 4. Graphical illustration of desirability and overlay plot (yellow area denotes the feasible region)

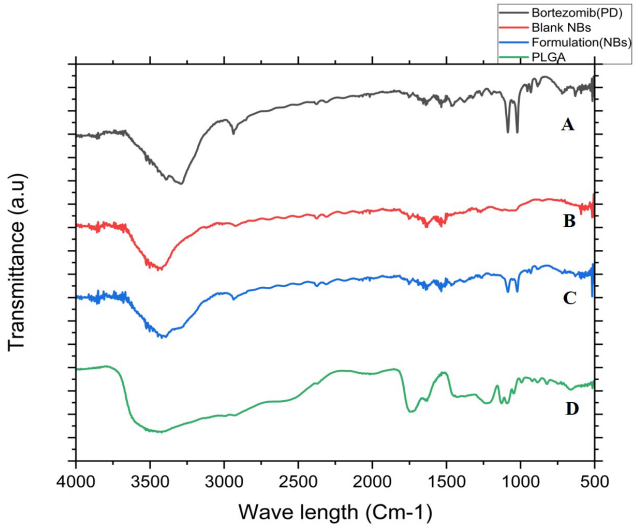

Figure 5. A) Overlay of FTIR analysis A: BTZ-PD (black line- pure drug); B: Blank NBs (red line-); C: Formulation NBs (Blue line- NB); D) PLGA (green line)

BTZ-PD: Bortezomib-pure drug, FTIR: Fourier transform infrared spectroscopy, NBs: Nanobubbles, PLGA: Poly(lactic-co-glycolic acid)

the oscillation of bubbles can trigger the shell to open, thereby aiding in the release of drugs. The study aligned with previous findings on NB stability under varying temperature conditions. The acoustic streaming flow generated by bubble oscillation regulates the movement of detached materials, which is influenced by both the radial excursion and the duration of the ultrasound pulse.[18]

Stability studies

BTZ-loaded NBs (NBs) underwent stability assessments at various storage conditions at 4 °C, at 25 °C, and at 40 °C immediately, one month, two months, and three months (Table 3). At 4 °C and 25 °C, minimal changes in drug content indicated robustness, with % EE showing slight variation, suggesting protection against degradation. However, a notable reduction in % EE occurred at elevated temperatures, where % EE was reduced to 62.41±3.90% from 65.12±2.54%, indicating structural disruption. Throughout the experiment, the PS of the formulation was less than 200 nm, and the ZP was around 29±2.29 mV, highlighting the stability and uniformity of BTZ NBs. Storage in a polyethylene pouch led to a faster drop in the number concentration compared to when stored in a glass bottle. Hydrogen bonding interactions were emphasized as critical factors in forming bulk NBs and their exceptional long-lasting stability.[17]

Pharmacokinetic studies

Figure 8 displayed the plasma concentration-time curve after drug administration in a 0.25% *w/v* sodium carboxymethyl cellulose solution and the optimized NBs, administered orally. Pharmacokinetic data in Table 4 revealed that the formulation exhibited significantly higher T_{max}, C_{max} ($p<0.001$**), AUC_0-24 ($p<0.001$**), and AUC_0-∞ ($p<0.001$**) values compared to the pure drug suspension at the prescribed dose. The bioanalytical chromatogram indicated drug retention time at 4.0 min and internal standard (Antipyrine) at 6.8 min (Supplementary Figure 3).

The optimized formulation reached a maximum level (C_{max}) 1.69 times higher, while the area under the curve (AUC_{0-t}) was 1.63 times higher than that of the free drug. *In vivo* studies revealed a progressive drug release from the NB preparation, with an

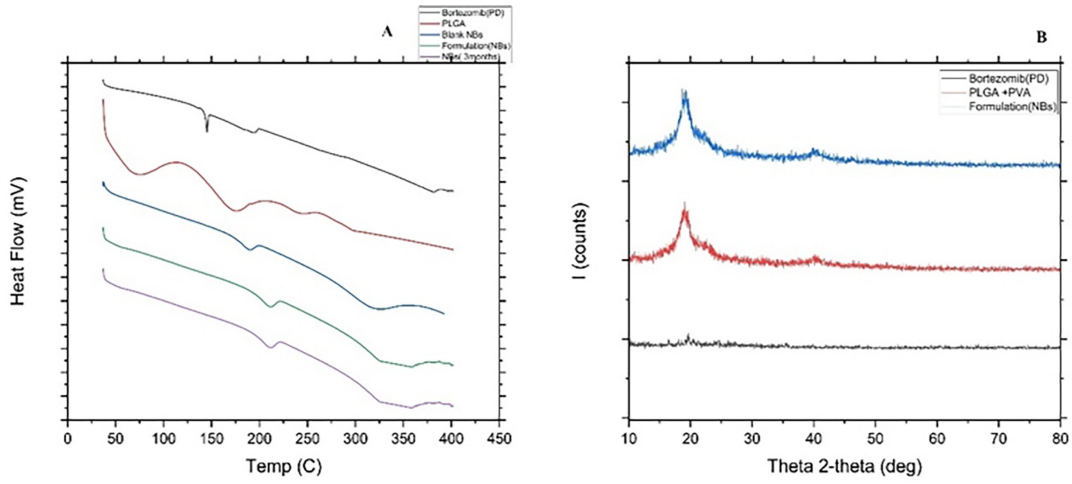

Figure 6. A) Overlay of DSC thermograms of i) BTZ-PD (black line); ii) PLGA (red line); iii) Black NBs (blue line); iv) Formulation (optimized NBs green line); v) Blanks NBs stored for 3 months (purple line); B) Overlay of XRD of i) BTZ-PD (black line); ii) PM (PLGA + PVA + drug-red line); iii) Formulation-NBs (blue line)

DSC: Differential scanning calorimetry, XRD: X-ray diffraction, BTZ-PD: Bortezomib-pure drug, PLGA: Poly(lactic-co-glycolic acid), NBs: Nanobubbles, PM: Physical mixture, PVA: Polyvinyl alcohol

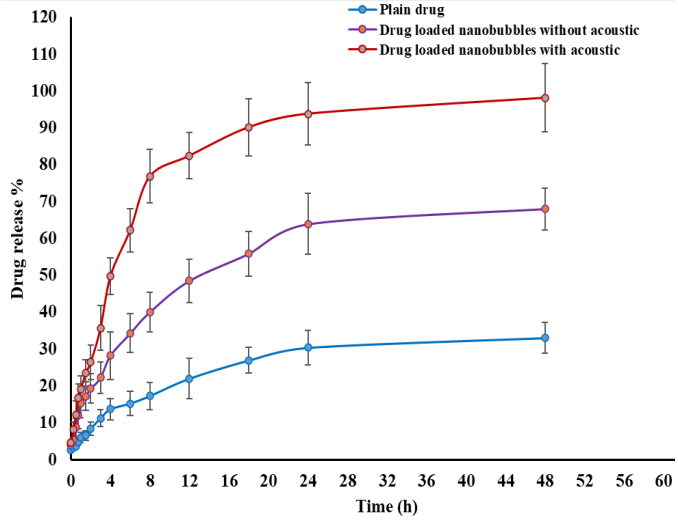

Figure 7. *In vitro* drug release of plain drug, drug-loaded NBs with and without ultrasound aid

NBs: Nanobubbles

extended half-life. The comparison of the data to the free drug shows that oral bioavailability has significantly improved. These findings suggested a notable improvement in oral bioavailability compared to the free drug. The enhanced bioavailability can be attributed to the increased drug circulation at the nanoscale and the improved penetration facilitated by the polymeric carrier system.

DISCUSSION

In this study, BTZ-loaded PLGA NBs were formulated using the solvent evaporation method, and optimization was conducted through the BBD. NBs are emerging as a formulation strategy because of their targeting ability. The process in which nanodroplets in perfluoro pentane transition from liquid to vapor upon exposure to ultrasound waves is called acoustic droplet vaporization.[24] Perfluoropentane undergoes a phase transition that turns nanodroplets into NBs with high US wave reflectivity. The echogenic qualities of perfluoropentane make it visible in ultrasonography images.[23]

The NBs were developed using PLGA polymer containing unbound carboxylic end chains, with perfluoropentane for the inner core and PLGA as the outer shell.[23] The term "ultrasound" describes mechanical vibrations or pressure waves that exhibit compressional and rarefactional pressure fluctuations and have frequencies equivalent to or higher than the human hearing threshold (20 kHz). Ultrasound effects primarily involve two mechanisms: cavitation and sonoporation. The cavitation effect plays a crucial role in reducing the size of bubbles, whereas the sonoporation effect facilitates the uptake of these reduced bubbles.[30] Hydrophobic interactions play a crucial role in bonding PLGA with the drug by forming NBs, while polyvinyl alcohol (PVA) acts as a stabilizing agent. PVA creates a protective coating around the NBs to uphold their stability. Due to its biocompatibility and biodegradability properties.

Combining ultrasound with NBs helps in drug localization while overcoming off-target effects. NBs utilizing PLGA have garnered attention for targeted drug delivery because of their unique physical and surface properties. PLGA is highly recommended in various medical applications, including sutures, bone implants, and sustained drug release systems, due to its biocompatibility and biodegradability properties.[14] The quadratic model suggested by the design was applied to PS, PdI, ZP, and % EE. Positive coefficients in the model indicated that an increase in associated variables led to higher drug

Table 3. Stability data of the NBs stored at various temperatures

Temperature	Months	PS (nm)	PdI	% EE
5±3 °C	Initial	186.90±13.9	0.146±0.042	66.12±1.48
	0.5	187.82±8.25	0.146±0.057	66.0±1.65
	1	188.38±9.10	0.148±0.044	65.09±3.92
	2	190.48±7.04	0.172±0.034	64.86±3.30
	3	191.26±8.34	0.164±0.037	63.19±3.66
25±2 °C	Initial	186.91±13.9	0.146±0.042	66.12±1.48
	0.5	187.26±2.16	0.149±0.066	66.06±2.85
	1	188.40±2.28	0.154±0.010	64.83±4.60
	2	192.68±4.98	0.180±0.041	63.37±2.89
	3	194.33±5.48	0.246±0.064	62.01±3.58
40±2 °C	Initial	186.89±13.9	0.146±0.042	66.12±1.48
	0.5	189.35±5.26	0.149±0.088	64.58±2.81
	1	194.13±6.06	0.218±0.032	62.22±2.16
	2	196.47±8.70	0.266±0.038	61.79±3.38
	3	199.26±9.52	0.296±0.046	60.10±3.60

PS: Particle size, PdI: Polydispersity index, EE: Encapsulation efficiency, NBs: Nanobubbles

Table 4. Pharmacokinetic parameters

Pharmacokinetic parameters	Pure drug	Drug-loaded NBs	Reference (Marketed)
C_{max} (ng/mL)	93.2±8.14	158.06±8.62	112.89±6.60
T_{max} (h)	6	6	0.5
Half-life (h)	47.70±6.16	52.81±8.40	26.05±6.63
AUC_{0-t} (ng. h/mL)	4382.38±174.28	7178.37±319.94	1380.40±89.94
Ke (h-1)	0.0145	0.0131	0.0266
MRT (h)	68.71±6.21	77.35±7.43	32.60±6.14

NBs: Nanobubbles, AUC: Area under the curve, MRT: Mean residence time

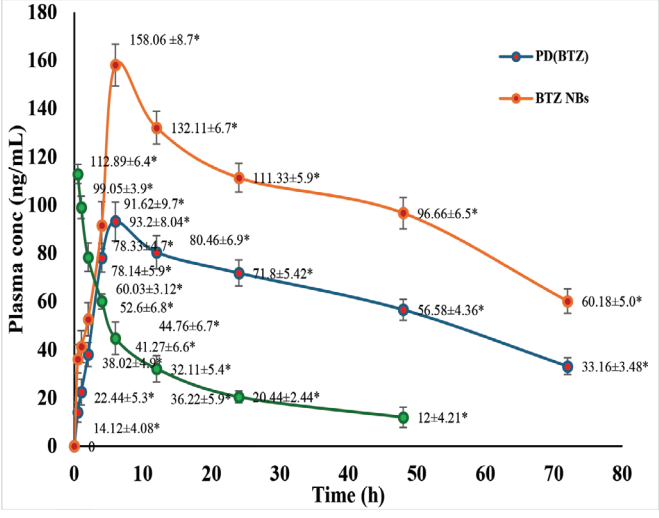

Figure 8. *In vivo* pharmacokinetic studies

entrapment. The concentration of the stabilizer has influenced PS, PdI, and ZP. However, when it came to % EE, a high stabilizer concentration led to a decrease in drug entrapment. PVA, as a stabilizer, has the potential to impede drug entrapment in NBs due to its competition for surface adsorption and the subsequent increase in solution viscosity. Moreover, PVA may hinder drug diffusion into NBs during the formation process. Furthermore, its destabilizing effects can result in premature rupture or aggregation, ultimately diminishing the efficiency of drug entrapment.[31] FTIR studies confirmed compatibility between the drug and excipients. DSC and XRD studies revealed no distinct drug peak in the formulation, indicating the absence of crystalline drug material.[10,28]

Electron microscopy helped in the direct visualization of NBs, assessment of their integrity, and analysis of their gas composition. SEM analysis displayed homogeneous, smooth, spherical NBs. Drug release occurred due to cavitation collapse induced by acoustic waves, disrupting NB structures and

enabling rapid delivery of medication. Ultrasound stability studies indicated the transformation of the gas core from nanodroplets to bubbles, known as acoustic droplet generation.[32] The study aligned with the previous findings on NB stability under varying temperature conditions. The temperature-dependent behaviour observed in PS, ZP, and entrapment emphasizes understanding NB characteristics in diverse environmental conditions for practical drug delivery applications. Different polymer materials submerged in NB dispersions exhibited varied effects on NB number concentrations due to hydrophobic interactions. *In vivo* studies in Wistar rats revealed gradual drug release from the formulation, leading to an increased half-life of the drug with high AUC. These findings indicated a significant improvement in the oral bioavailability of the chosen medicine when using NBs compared to the free drug.[32] The improved bioavailability of NBs with PLGA is achieved through various mechanisms. These include better drug encapsulation within PLGA nanoparticles, longer circulation time due to the protective coating of PLGA, and increased uptake by target cells or tissue. As a result, these mechanisms contribute to enhanced therapeutic outcomes. By enhancing tumor penetration and drug release, US-driven cavitation helps to precisely and site-specifically activate drugs. By combining ultrasound with NBs, drug localization can be achieved while mitigating off-target side effects.Optimizing stability, dose, and safety remains difficult, even if it could help reduce side effects and counteract medication resistance. Clinical acceptance depends on standardizing US characteristics among different modalities. Rigorous trials, translational research, and regulatory validation collectively define the successful incorporation of treatments. To guarantee scalability, large-scale manufacturing has to be cost-effective and efficient. Promoting this technology requires a multidisciplinary strategy to link actual uses with experimental results.

CONCLUSION

This research introduced an innovative approach to improving the solubility of BTZ NBs. By conducting a thorough investigation, the study demonstrated that NBs significantly enhanced the release of the drug, indicating their potential as a new and smart delivery system. Response surface methodology ensured a precise control over the size distribution, resulting in improved uniformity. Additionally, drug-loaded NBs exhibited exceptional stability and dissolvability in the gastrointestinal tract compared to the traditional drug suspensions, suggesting a longer drug half-life and increased effectiveness. These findings highlighted a promising role of PLGA NBs in ultrasound-responsive formulations for cancer therapy, offering notable benefits such as faster dissolution rates, sustained, targeted drug release, and improved oral bioavailability.

Ethics

Ethics Committee Approval: The animal protocol was designed and approved by the Institutional Animal Ethics Committee of Teegala Ram Reddy College of Pharmacy (approval number: 1447/PO/Re/S/11/CPCSEA-97/A, dated: 17.02.2024).

Informed Consent: Not required.

Footnotes

Authorship Contributions

Concept: N.K., K.K.Y., Design: N.K., K.M., K.K.Y., Data Collection or Processing: N.K., Analysis or Interpretation: N.K., Literature Search: N.K., K.M., Writing: N.K., K.M.

Conflict of Interest: The authors declare no conflicts of interest.

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Supplementary Table 1. Physicochemical and pharmacokinetic properties of bortezomib

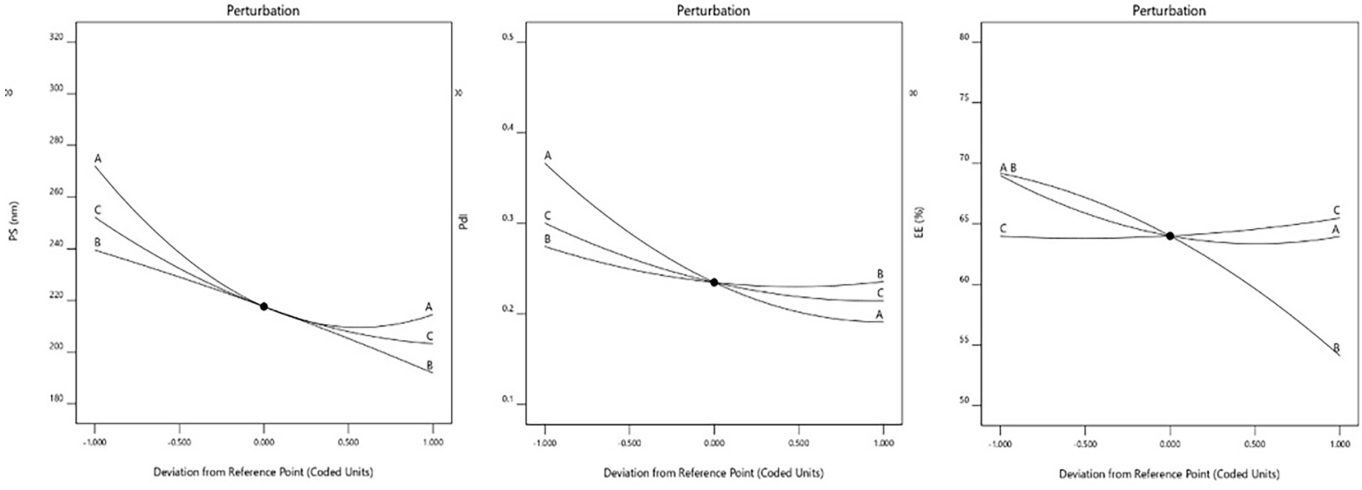
Molecular structure	
IUPAC name	[(1R)-3-methyl-1-[[(2S)-3-phenyl-2-(pyrazine-2-carbonylamino) propanoyl] amino]butyl]boronic acid
Molecular weight	384.243
logP	0.89 (ALOGPS)
logP	1.53 (ChemAxon)
pKa (strongest acidic)	13.04 (ChemAxon)
pKa (strongest basic)	–0.7 (ChemAxon)
Water solubility	0.0532 mg/mL
Physiological charge	0

IUPAC: International Union of Pure and Applied Chemistry

Supplementary Table 2. FTIR spectral data with wave number and the corresponding groups

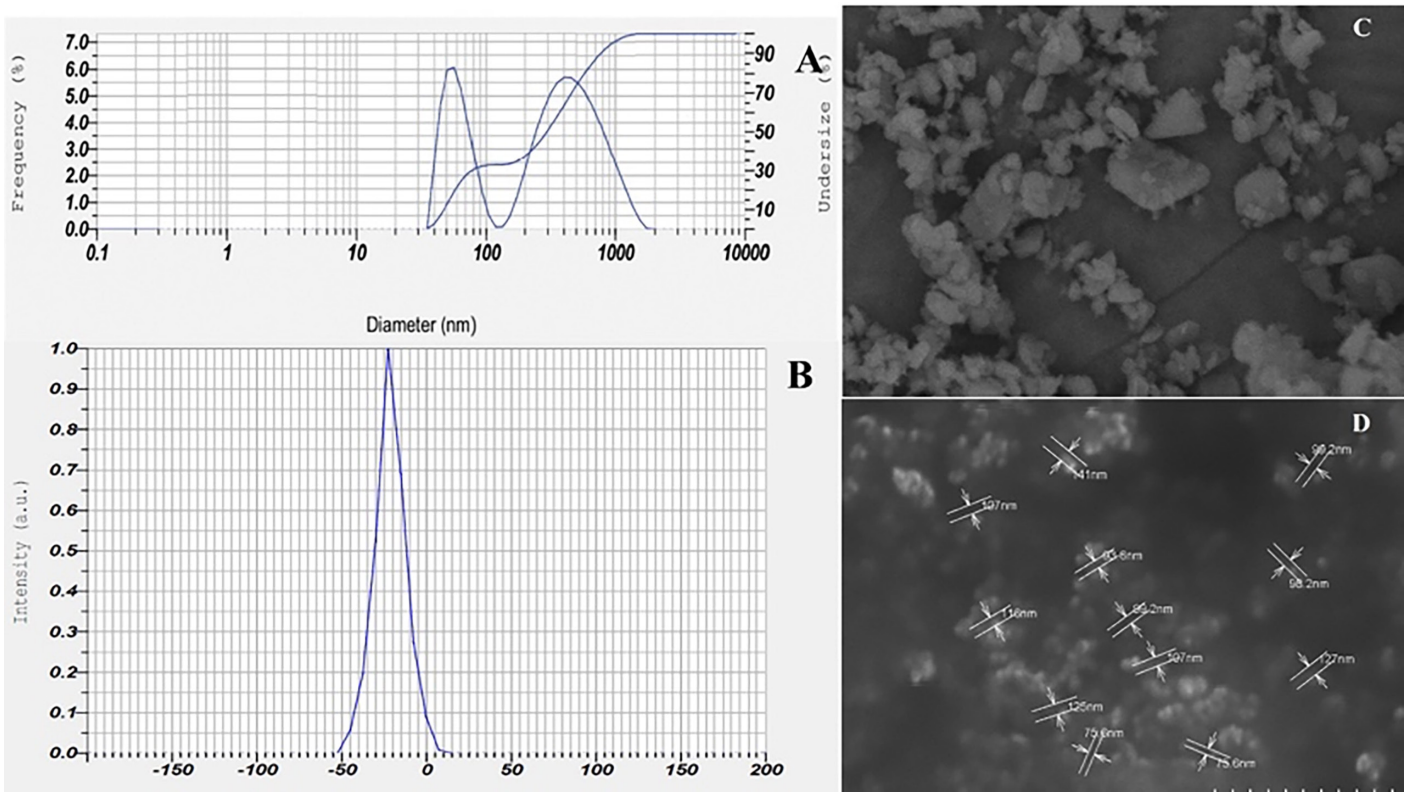
Wave number (cm^{-1})	Corresponding group in pure drug	Wave number (cm-1)	Corresponding group in pure drug
3446.9	O-H stretching, typical in alcohols and phenols	1394	B-O stretching
3294.18	O-H stretching in B-O-H group of BTZ	1261.49	C-N stretching, potentially from amines or amides.
2937.68	C-H stretching, suggesting alkanes or methyl groups.	1193.98	C-O stretching (ethers or esters)
1747.57	C=O stretching of carbonyl groups (ketones or aldehydes)	1085.96	C-O stretching (alcohols, ethers, or esters).
1663	C=C stretching, typical of alkenes or aromatic compounds	1022	C-N stretching (amines or amides)
1523.9	amide C=O stretching	885.36	C-H bending (alkanes or methyl groups)
1460	C-H bending (alkanes or methyl groups)	719.47	C-H bending (alkanes or methyl groups)

BTZ: Bortezomib, FTIR: Fourier transform infrared spectroscopy

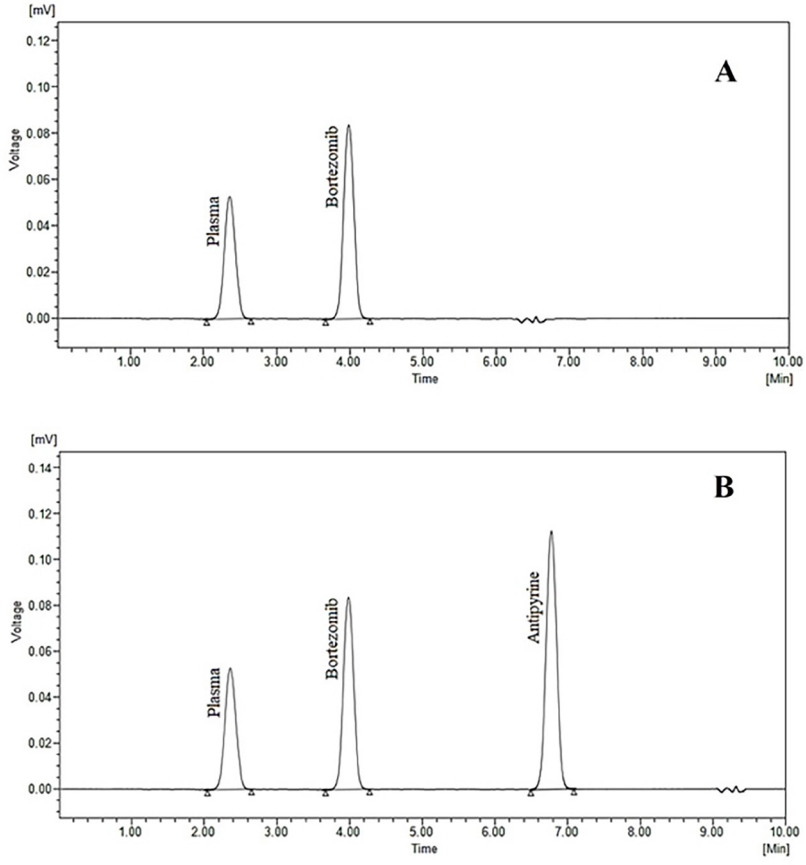


Supplementary Figure 1. Perturbation plots of PS, Pdl, and EE

PS: Particle size, Pdl: Polydispersity index, EE: Encapsulation efficiency



Supplementary Figure 2. A) Particle size; B) ZP; C) SEM of plain drug; D) SEM of optimized NBs
EE: Encapsulation efficiency, ZP: Zeta potential, SEM: Scanning electron microscopy



Supplementary Figure 3. Bioanalytical method development of the drug in plasma (A), and drug and internal standard in plasma (B)

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ORIGINAL ARTICLE

Evaluation of Corporate Social Responsibility Practices in the Turkish Pharmaceutical Industry

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ABSTRACT

Objectives: Corporate social responsibility (CSR) is defined as companies voluntarily taking action with their resources to help solve some of the social, economic, and environmental problems of the society in which they operate. This study aimed to reveal the current status of CSR projects in the pharmaceutical industry, which are strategically important on a global scale.

Materials and Methods: The study was conducted between June and December 2019 using a structured online survey. The form included both multiple-choice and open-ended questions. This survey yielded both qualitative and quantitative data about the structure, size, and products of the companies, as well as details of CSR projects.

Results: A total of 60 companies participated in the study. Our survey results indicated that 83.33% of the pharmaceutical companies undertook CSR projects, and there was no statistically significant difference in the number of projects undertaken according to either the type of products the companies marketed or the companies' country of origin ($p>0.05$). It was found there were statistically significant differences with other factors such as project fields, the number of projects over the years, and the responsible department ($p<0.05$). When we examined the details of specific CSR projects, we observed that some companies, regardless of their national or multinational position, undertook sustainable projects that involved a broader array of stakeholders and appealed to various shareholder groups. Some companies undertook studies for patients, patient relatives, or society based on philanthropic activities or short-term social projects on local issues, instead of long-term projects within the scope of CSR initiatives.

Conclusion: Pharmaceutical companies carry out CSR projects as an important public relations activity in terms of reaching their stakeholders.

Keywords: Pharmaceutical industry, corporate social responsibility, social responsibility, Türkiye

INTRODUCTION

Understanding Corporate social responsibility (CSR) has gained importance worldwide due to such developments as globalization, increasing social awareness, and the challenges of competition.[1,2]

There is no single, universal definition of CSR, since the term undergoes changes in response to dynamic national and international social and economic conditions. The concept of CSR, which encompasses responsibility as well as social responsibility, covers many domains, including human rights, transparency and accountability, occupational health and safety, business ethics, ethical working perception, environmental protection, sustainable development, and the fight against corruption.[3] Furthermore, CSR projects differ within the framework of the specific conditions, values, and economic status of the countries in which they are conducted, as well

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as the expectations of stakeholders.[4] CSRs and public relations (PR) have a lot in common, such as public welfare, and they are strategically important to firms' social roles.[5,6]

Activities related to CSR conducted by the public, private sector, or non-governmental organizations are also applicable in the healthcare sector. Yet for purposes of both sincerity and accuracy, it is vitally important that studies conducted in this field be constrained by ethical limits. Among other things, the ethical framework must be constructed to prioritize the social good rather than advertising.[7] Social responsibility in healthcare was first discussed at the World Health Organization's 4th Health Promotion Conference in 1997. The "Declaration of the International Bioethics Committee" by the United Nations Educational, Scientific, and Cultural Organization is also concerned with social responsibility and health, emphasizing that the most important goal of administrations and all societal sectors is to promote public health and social development.[7] Nevertheless, published studies contain very limited information about the CSR projects undertaken by the pharmaceutical industry, despite its being one of the world's most important and strategic sectors for human welfare.[8-12]

According to the research, surveys examining the CSR projects of pharmaceutical companies found that, since the projects are undertaken voluntarily, each company decides on the scope of its own CSR projects independently. The leading areas of these projects include innovative medicine development; access to medicine and pricing strategies; environmental initiatives such as reducing ecological footprint and managing pharmaceutical waste; social practices focusing on employee well-being, equality, and fair working conditions; philanthropic efforts including donations and educational support; and ethical commitments to transparency and risk management.[8,10-12]

Controversies have been generated by the conflict of interest arising from the industry's dual roles. As a developer and supplier of innovative and life-saving medicines, the pharmaceutical industry has both an entrepreneurial role and a healing role that carry responsibilities to society. The controversies mainly concern product recall activities; direct-to-consumer advertising and industry-supported education; pricing, access to pharmaceuticals; innovation; and patents.[9] Some of the criticisms of the industry are about unethical animal testing and unethical life-saving pharmaceuticals that are unaffordable for the poor.[13] The industry is under great pressure to reduce costs, particularly now that patents for high-tech and therapeutically important products have expired and product safety reviews have increased.[8,12,14] The role of CSR projects in improving the brand image is important.[15]

In response to such criticism, pharmaceutical companies have significantly increased their CSR efforts over the past two decades, particularly in low and middle-income countries that have the highest global incidence of disease.[13] In developing countries, these companies actively undertake the necessary activities to fight against Human Immunodeficiency Virus/ Acquired Immune Deficiency Syndrome and other epidemics and pandemics, and to promote social welfare.[13] Pharmaceutical companies have also been criticized for using CSR to restore compromised reputations or to reverse public beliefs about the companies' unethical business practices.[13]

The pharmaceutical industry is the third largest industry in the world, and according to 2018 data, Türkiye ranks 17th in the world's pharmaceutical markets.[16] As part of the global pharmaceutical market, pharmaceutical companies operating in Türkiye conduct CSR projects. However, there have been no scientific studies investigating Turkish pharmaceutical companies and their CSR projects.

Public welfare, the common point of CSR and PR, is also important to the pharmaceutical industry. Moreover, no recent study has examined the current status of CSR, which is significant for sustainability within the pharmaceutical industry in Türkiye. In this study, we aimed to reveal the current status of CSR projects in the pharmaceutical industry and to determine the relationship between variables such as the companies' profiles, whether national or multinational, product groups in the market, and their structuring with CSR projects.

MATERIALS AND METHODS

In this study, a descriptive analysis was conducted to reveal the current status of the CSR projects of pharmaceutical companies in Türkiye.

Survey design and data collection

To ensure a comprehensive evaluation, 21 questions were developed by considering topics frequently addressed in the literature and structures that emerged in previous similar studies.[1,5,8,11-14] In addition, the questions were clarified in line with expert opinions and organized to be suitable for the pharmaceutical industry. In this context, simplifications were made to ensure that the participants could understand the questions. Before implementation, the survey was finalized based on expert feedback. This survey yielded both qualitative and quantitative data about the structure, size, and products of the companies, as well as details of CSR projects between 2009 and 2019.

CSR categories have been determined according to the categories addressed in similar studies in the literature. CSR projects were categorized as belonging to the following areas: health, education, environment, culture and art, sports, philanthropy, science, and other. Responses to the survey questions yielded additional information about the CSR projects, including project names, start and end dates, target audience, *etc*. This information was classified according to the CSR project groups. Projects undertaken before 2009 or having uncertain dates were excluded.

To investigate the pharmaceutical industry's status in Türkiye, 110 pharmaceutical companies were identified using membership lists of the following industry associations: The Association of Research-Based Pharmaceutical Companies (AIFD); Pharmaceutical Manufacturers Association of Türkiye (IEIS); and Turkish Pharmaceutical Industry Association (TISD). Each of the 110 pharmaceutical companies was a member

of at least one of the above-mentioned associations. The survey was shared with identified companies through email. Participants were informed about the purpose of the study, data confidentiality, and the voluntary nature of participation. Those who gave their consent participated in the survey. Data collection was completed between June and December 2019.

Ethical approval

This study was approved by the Ethics Commission of Hacettepe University (approval number: E.563072, dated: 25.04.2019).

Statistical analysis

Statistical analysis was conducted using Statistical Package for the Social Sciences for Windows (Version 23.0; Armonk, NY: IBM Corp., USA). In addition to descriptive statistics, non-parametric tests such as the chi-square test, Fisher's exact test, and Mann-Whitney U test were conducted to compare the groups. The confidence level was set as 95%, and the level of significance was determined as 0.05.

RESULTS

A total of 60 pharmaceutical companies participated in the survey (54.5% of the 110 companies identified). Some of the companies belong to more than one of the above-mentioned industry associations. Of the participating companies, 37.50% are members of AIFD, 33.75% are members of IEIS, 8.75% are members of TISD, and 20% are also members of additional organizations. Other basic characteristics of the companies are given in Table 1.

With respect to employment, it was found that 101-500 staff were employed with a rate of 38.33%, and a total of 212,677 staff were employed in the pharmaceutical companies that participated in the survey. The total number of products produced by the participating companies was 6,717 (Table 1).

The main product groups for which the companies were the manufacturers or the license-holders were then evaluated. The research found that conventional products were in the first place with 52.38%. In second place were biotechnological products, which were produced at a rate of 26.67%. In the third place were biological products, which had production and licensing rates of 15.24%. It was found that 5.71% of the companies also had products from other groups.

The Mann-Whitney U test was conducted to compare the number of CSR projects and the companies' characteristics, their profile as national or multinational, and their organizational structure, more specifically, whether the company was structured to publicize its CSR activities (Table 2).

As seen in Table 2, there is no statistically significant difference between the number of CSR projects undertaken by national and multinational companies ($p>0.05$). However, there is a statistically significant difference between the number of CSR projects undertaken by companies with and without a corporate communications department ($p<0.05$). Companies with a corporate communications department were found to

Table 1. Characteristics of the companies

	Number of companies, n	%
Characteristics of the companies		
Local	29	48.33
Multinational	31	51.67
Total	60	100
Number of employees		
100 and below	14	23.33
101-500	23	38.33
501-1000	13	21.67
Over 1000	10	16.67
Total	60	100
Number of products		
100 and below	38	63.33
101-200	10	16.67
201-300	5	8.33
Over 300	5	8.33
N/A	2	3.33
Total	60	100

N/A: Not applicable

Table 2. Comparison between the number of CSR projects and companies' characteristics as national or multinational and organizational structure

Characteristics	Number of projects carried out between 2009-2019			
	Number (n)	Mean ± SD	Median (min.-max.)	Test value and *p*
Companies' characteristics				
National company	29	5.28±6.135	3.00 (0-25)	z=-1.033 *p*=0.302
Multinational Company	31	9.45±19.358	4.00 (0-107)	
Availability of the corporate communications department				
No corporate communications department	24	3.13±4.446	2.00 (0-20)	z=-3.434 *p*=0.001
Has a corporate communications department	36	10.31±18.016	6.00 (0-107)	

CSR: Corporate social responsibility, SD: Standard deviation, min.-max.: Minimum-maximum

have a higher number of CSR projects than those without that department.

For the comparison between the number of CSR projects and product groups, Fisher's exact tests and chi-square analyses were conducted (Table 3).

The research found no statistically significant difference among conventional, biotechnological, and biological medicine producers ($p>0.05$). Although there was no statistically significant difference, it was determined that conventional medicine producers initiated projects mainly in the fields of health and education and occasionally in other fields (specifically in ethics and business ethics); biotechnological medicine producers initiated projects mainly in the fields of health and education, and occasionally in other fields (specifically in ethics and business ethics). Biological medicine producers mainly project in the fields of health, education, and volunteer work, but did not initiate projects in other fields (ethics and business ethics, informatics) (Table 3).

Companies reported that the number of projects between 2009, 2019 totaled 381. When examining the CSR projects by year, it was found that their number has increased significantly since 2012 (Figure 1).

The year in which the most projects were undertaken was 2018 (20.7% of all projects), and the next highest year was 2017 (16.8% of all projects undertaken). It was determined that at least 1% of the projects were undertaken in 2009. The result of the single-sample chi-square test is presented in Table 4.

There is a statistically significant difference in the number of CSR projects undertaken by the companies each year ($p<0.05$). The number of CSR projects undertaken by companies increased until 2018 (Table 4).

Some of the projects concern more than one area, and the distribution of the project areas has been examined over the years. During the evaluation process, due to the small number of projects between 2009 and 2012, the data from these years was combined in order to make a statistical analysis. The 381 projects cover a total of 530 fields of activity. Table 5 presents the results of the Chi-square test and Fisher's exact test regarding CSR project areas over the years.

Table 3. CSR project groups and companies' product groups

Fields of CSR projects	No conventional products		Has conventional products		*p*	No biotech products		Has biotech products		*p*	No biotech products		Has biotech products		*p*
	n	%	n	%		n	%	n	%		n	%	n	%	
Health															
Yes	2	66.7	35	74.5	1.000	19	70.4	18	78.3	0.526	28	73.7	9	75.0	1.000
No	1	33.3	12	25.5		8	29.6	5	21.7		10	26.3	3	25.0	
Education															
Yes	1	33.3	35	74.5	0.186	18	66.7	18	78.3	0.363	27	71.1	9	75.0	1.000
No	2	66.7	12	25.5		9	33.3	5	21.7		11	28.9	3	25.0	
Environment															
Yes	-	-	14	29.8	0.550	7	25.9	7	30.4	0.723	10	26.3	4	33.3	0.718
No	3	100.0	33	70.2		20	74.1	16	69.6		28	73.7	8	66.7	
Culture-arts															
Yes	1	33.3	6	12.8	0.370	4	14.8	3	13.0	1.000	5	13.2	2	16.7	1.000
No	2	66.7	41	87.2		23	85.2	20	87.0		33	86.8	10	83.3	
Sports															
Yes	1	33.3	11	23.4	1.000	8	29.6	4	17.4	0.313	10	26.3	2	16.7	0.705
No	2	66.7	36	76.6		19	70.4	19	82.6		28	73.7	10	83.3	
Volunteer work															
Yes	2	66.7	30	63.8	1.000	18	66.7	14	60.9	0.670	23	60.5	9	75.0	0.497
No	1	33.3	17	36.2		9	33.3	9	39.1		15	39.5	3	25.0	
Science															
Yes	2	66.7	8	17.0	0.098	3	11.1	7	30.4	0.155	7	18.4	3	25.0	0.686
No	1	33.3	39	83.0		24	88.9	16	69.6		31	81.6	9	75.0	

CSR: Corporate social responsibility

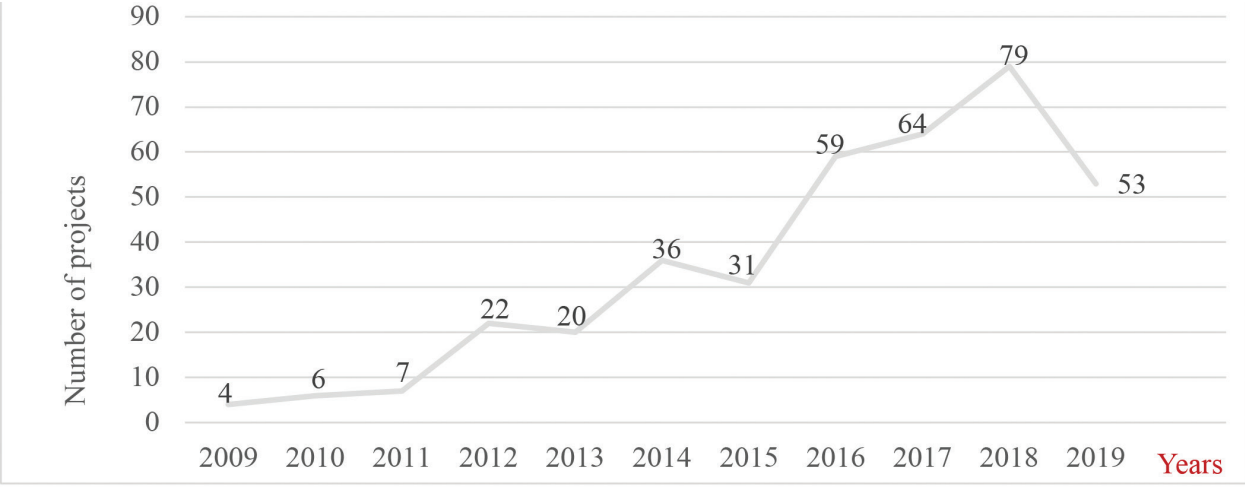

Figure 1. The number of CSR projects over the years
CSR: Corporate social responsibility

Table 4. The changes in the number of CSR projects over the years

Years	Conducted CSR projects		
	Number (n)	Percentage (%)	Test value and *p*
2009	4	1.0	
2010	6	1.6	
2011	7	1.8	
2012	22	5.8	
2013	20	5.2	
2014	36	9.4	χ^2=192,646 p<0.001
2015	31	8.1	
2016	59	15.5	
2017	64	16.8	
2018	79	20.7	
2019	53	13.9	

*Percentage calculations are based on the total number of CSR projects (n=381). CSR: Corporate social responsibility

In Table 5, it can be observed that there is a statistically significant difference in terms of culture and arts (p<0.05). However, there is no statistically significant difference in terms of other fields of CSR projects (health, education, environmental issues, culture and arts, sports, philanthropy, science, other) by years (p>0.05).

Table 6 presents the results of the chi-square test and Fisher's exact test, revealing the difference between methods used to announce their CSR projects and companies' profiles, as being either national or multinational.

There is a statistically significant difference between the number of press releases and whether the companies are national or multinational (p<0.05). We determined that 65.5% of multinational companies made press releases about CSR projects (Table 6). The companies' profiles as either national or multinational show no statistically significant difference between not making announcements and announcing CSR projects by methods other than press releases (by sustainability reports, annual reports, or internet/social media) (p>0.05).

DISCUSSION

The fact that CSR campaigns are more visible, and their participation in society, has effects on the image of the company.[17] At this point, proper planning of CSR strategies is significant for the PRs of pharmaceutical companies.[18] Within the scope of this study, this study aimed to determine the current situation of CSRs in Türkiye, CSRs carried out by pharmaceutical companies that have an important place in society.

Table 5. CSR project areas by years

Variables		2012 and before	2013	2014	2015	2016	2017	2018	2019	p
Health	n	8	7	16	10	23	24	27	11	0.183
	%	20.5	35.0	44.4	32.3	39.0	37.5	34.2	20.8	
Education*	n	17	8	12	12	15	20	16	16	0.201
	%	43.6	40.0	33.3	38.7	25.4	31.3	20.3	30.2	
Environmental issues	n	9	2	2	3	5	4	9	4	0.291
	%	23.1	10.0	5.6	9.7	8.5	6.3	11.4	7.5	
Culture-arts	n	2	5	2	4	2	3	2	3	0.035
	%	5.1	25.0	5.6	12.9	3.4	4.7	2.5	5.7	
Sports	n	-	1	1	-	-	-	1	2	0.224
	%	-	5.0	2.8	-	-	-	1.3	3.8	
Philanthropy	n	16	6	17	14	31	30	36	27	0.779
	%	41.0	30.0	47.2	45.2	52.5	46.9	45.6	50.9	
Science	n	3	2	2	1	1	3	3	7	0.243
	%	7.7	10.0	5.6	3.2	1.7	4.7	3.8	13.2	
Other**	n	2	2	2	3	1	2	10	1	0.091
	%	5.1	10.0	5.6	9.7	1.7	3.1	12.7	1.9	

*Projects in the field of education; it includes training for students in primary and secondary schools, universities, patients, their relatives, and health professionals.
**Other: Support projects for women workers/producers/cooperatives, activities for children in need of protection, support for mothers and children in the shelter, various informative activities, awareness programs, gender equality awareness program, protection of women's rights, etc.
CSR: Corporate social responsibility

Table 6. The announcement methods of CSR projects according to companies' profiles as national or multinational

The method of publicizing CSR projects	Local company		Multinational company		
	Number (n)	Percentage (%)	Number (n)	Percentage (%)	*p*
Sustainability reports	3	14.3	9	31.0	0.171
Annual reports	7	33.3	7	24.1	0.475
Internet/social media	17	81.0	25	86.2	0.706
Press releases	7	33.3	19	65.5	0.025
No announcement	2	9.5	2	6.9	1.000

CSR: Corporate social responsibility

In Türkiye, CSR projects, which started later than in many other countries, have developed in recent years. In this study, it was determined that 83.33% of the survey respondents from the pharmaceutical companies operating in Türkiye undertook a CSR project between 2009 and 2019.

The main CSR project area undertaken by pharmaceutical companies operating in Türkiye was philanthropy, followed by health and education fields, while the fewest projects were undertaken in the field of sports. It is thought that Türkiye's socio-cultural structure and social values explain why philanthropic areas are predominant among its companies' CSR projects. Thus, it was found that many health-related projects but relatively few sports-related projects were undertaken. These results agree with those of similar studies in the literature.[19-25] When examining the literature on corporate CSR projects in different sectors of Türkiye, with particular attention to philanthropy, it may be observed that the most common CSR project areas are health, environment, education, culture, arts, and sports.[19-25]

Every country's pharmaceutical industry is highly affected by regulatory requirements, government policies, social and political pressures, and economic constraints. It is therefore expected that characteristics of CSR projects, including the areas in which they are predominantly undertaken, will vary according to the pharmaceutical companies' countries of origin, specifically the cultures, political, and regulatory environments

of those countries.[10] In this study, a statistically significant difference was found between national and multinational companies concerning the number of CSR projects they undertook. It is evident from the available literature that some studies found differences in CSR practices related to companies' profiles as either national or multinational, whereas others found no remarkable differences.[10,26-30] In research conducted on companies representing European Union countries, Japan, the USA, and Canada in order to determine the CSR approaches of pharmaceutical companies in Canada, no difference was determined in terms of origin country. In a study conducted on the pharmaceutical industry in China, it was determined that foreign-owned pharmaceutical companies generally had better CSR; whereas privately-owned Chinese pharmaceutical companies participated in CSR at the lowest levels.[27]

This research found a significant difference between CSR activity and company structure. There was a statistically significant difference in the number of CSR projects undertaken by companies that have a corporate communications department as compared to companies without a dedicated corporate communications department. It is known that the world's leading pharmaceutical companies have communications departments, and that communicating the company's CSR activities has become those departments' primary focus.[13,31-33] In the past, companies assigned responsibility for CSR projects to their human resources or legal departments; today, the majority of the world's top 20 pharmaceutical companies assign these responsibilities to their communications or PRs departments.[31] Corporate communication units are typically responsible for establishing, implementing, and supervising communication strategies, and they therefore play a key role in broadcasting a company's CSR activities.[32] In the absence of such communication, it is not possible for studies of CSR to obtain the data needed to achieve their goals.[33] In a study conducted on multinational pharmaceutical companies in the USA, it was determined that CSR activities are managed differently: 50% of the companies have a department specifically for CSR, and all of them have a monitoring board or group specifically for that.[13] In addition, it is necessary to get support from PRs efforts to ensure the balance between the scope of the work and the activities carried out.[15]

This study revealed that there has been a noteworthy increase in the number of CSR projects since 2012, with a parallel development of CSR activities in Türkiye. The year in which the greatest number of projects was undertaken by companies was 2018, with a rate of 20.7%. The number of projects in 2019 was lower than in 2018. In this context, it has been observed that the number of newly started projects in 2017 and 2018 is high; however, some of the projects started in these years are continued in the following years as they are continuous projects. For this reason, it was understood that in 2019, the number of newly started projects, in addition to the ongoing projects, lagged behind the previous years. In 2009, it was understood that the fewest projects were undertaken, with only 1% of projects initiated. There is a statistically significant difference in the number of CSR projects undertaken by companies across different years. In this context, it is an inference supported by the literature pertaining to recent CSR efforts by international institutions and organizations.[11,34-37] CSR studies have developed over the years. At the Lisbon Summit held in 2000, globalization and technological developments were discussed.[11] After this summit, the Green Paper prepared by the Council of Europe was published in 2001. Since that date, the European Commission has issued documents setting forth basic policies and has continued to support the CSR agenda through publications designed to raise awareness.[11,35,36] In 2000, the "Global Principles Agreement" was published by the United Nations with recommendations entitled, "Multinational Enterprises General Principles" by the Organisation for Economic Cooperation and Development; and in 2003, "Millennium Development Goals" were formulated within the scope of the United Nations Development Programme.[37] International standards and declarations published by international institutions in the field of CSR are guiding and encouraging companies to engage in CSR practices.[34] Over the years, this issue has gained importance internationally. More systematic progress in this area can be achieved by bringing together industry representatives and authorities to determine roadmaps, considering the differences in practices in the pharmaceutical industry discussed in the results of this study.

CSR projects need to be publicized; however. This study found that multinational companies issued press releases about CSR projects at a rate of 65.5%. The literature contains several studies on the methods of announcing CSR projects.[10,38-42] Following CSR activities on websites and social media is critical in terms of PRs.[15] In a study examining CSR communications on companies' websites, it was found that Turkish companies use their websites for that purpose less frequently than do companies of Western countries, including the UK and the USA.[38] Another study conducted on multinational pharmaceutical companies in the USA found that those companies announced their CSR projects on their websites.[13] However, local pharmaceutical companies in Slovakia had communication problems due to insufficient use of communication channels, whereas large foreign pharmaceutical companies had far better CSR communication.[39]

Study limitations

The findings of this study were based on self-reporting by the companies. Some questions were left unanswered for reasons related to a given company's organizational or management policies. In particular, questions about the budget and cost were left unanswered because the release of such information was prohibited by the company's confidentiality policies. Therefore, questions about the budget could not be evaluated.

In addition, detailed answers were not given to questions that required comprehensive information about some projects, because detailed records were not kept of the CSR projects in the past years. For this reason, the analyses were made with limited quantitative data.

In this study, evaluating the reports and answering the questions did not allow for a determination of whether the

participants' evaluations were subjective. This situation can be considered a source of bias. The findings may have been biased due to the participants' subjective evaluations and recall biases. Additionally, since the study only evaluated responses from participants from the companies that voluntarily agreed to participate, this may have limited the generalizability of the data.

CONCLUSION

Companies in the pharmaceutical sector are manifesting interest in CSR and engaging in CSR projects, as are companies in other sectors, regardless of distinctions. However, it is understood that certain standards should be met, in line with the answers given by the companies, in terms of carrying out and announcing the activities.

Within the scope of this study, we concluded that to develop the Turkish Pharmaceutical Industry's CSR to disseminate information and increase awareness of CSR practices, university courses could be effective. In particular, we recommend expanding the undergraduate curriculum for occupational groups working in the pharmaceutical industry by adding courses on CSR's theoretical aspects and their practical applications. This addition to undergraduate education would prepare students to contribute to the development of existing programs. The curriculum—like all other activities of the pharmaceutical industry—would need to be developed by international standards and guidelines in the field of CSR. It will also be important to integrate the understanding of CSR into companies' management strateg. Within the framework of transparency, measurability, accountability, and sustainability principles, it should be possible to systematically undertake CSR projects and report on these activities within the scope of long-term strategic goals. Legal regulations governing CSR projects will also be important in this respect. Scientific activities held at the academic level, including articles, congresses, workshops, panels, and symposia, will complement the awareness of CSR through the work of NGOs, public institutions, and the public itself. We recommend emphasizing that CSR projects undertaken within the pharmaceutical sector make an important social contribution. Furthermore, these projects are important for achieving the Sustainable Development Goals. In this regard, to reveal the relationships between CSR, corporate practices, and sustainability efforts in the pharmaceutical sector, future studies should be planned across different contexts and stakeholder perspectives.

Ethics

Ethics Committee Approval: This study was approved by the Ethics Committee of Hacettepe University (approval number: E.563072, dated: 25.04.2019).

Informed Consent: Participants provided informed consent, as the survey introduction explained the study and clarified that participation was voluntary.

Footnotes

Authorship Contributions

Concept: S.A.S., B.S.Ş., S.Y., Design: S.A.S., B.S.Ş., S.Y., Data Collection or Processing: S.A.S., Analysis or Interpretation: S.A.S., B.S.Ş., Literature Search: S.A.S., B.S.Ş., S.Y., Writing: S.A.S., B.S.Ş., S.Y.

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ORIGINAL ARTICLE

Comparison of *In Vitro* and *In Vivo* Dissolution of Norvir® Oral Powder: *In Vivo* Relevance of a too Rapid *In Vitro* Dissolution Test

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ABSTRACT

Objectives: Norvir® oral powder [ritonavir (RTV)] employs polyvinylpyrrolidone/vinyl acetate as the polymer to formulate an amorphous solid dispersion. Its oral absolute bioavailability is 70% in the fasted state, and it has negative food effects. The aim of this study was to perform in vitro dissolution of Norvir® powder and Wagner-Nelson deconvolution of *in vivo* data under fasted, moderate fat, and high fat conditions in order to elucidate the relevance of *in vitro* dissolution testing.

Materials and Methods: *In vitro* dissolution of Norvir® oral powder was conducted, and the human pharmacokinetic data of Norvir® powder were obtained from literature, under fasted, moderate fat, and high fat conditions. Wagner-Nelson deconvolutions were performed. The absolute fraction absorbed (F_a) profiles were compared to the *in vitro* dissolution (F_d) profiles. Levy-Polli plot analysis was also conducted. For each pharmacokinetic condition, a scale factor was estimated to approximate the extent to which *in vitro* dissolution needed to be slowed down to mimic *in vivo* dissolution.

Results: Qualitatively, there was a large difference between *in vitro* and *in vivo* dissolution. *In vitro* dissolution showed 98% release in 5 minutes. Meanwhile, from Wagner-Nelson analysis, only 5.5% of the drug dissolved (and absorbed) *in vivo* in 5 min under fasted conditions. It was not until 2 hr that 49% of the RTV dose dissolved (and was absorbed) *in vivo*. *In vivo*, moderate fat and high fat conditions were even slower in producing a certain effect. The Levy-Polli plot exhibited a "reverse-L" profile. It was concluded that such rapid *in vitro* dissolution did not mimic the *in vivo* dissolution of RTV. *In vitro* dissolution needed to be slowed by 100-fold for fasting.

Conclusion: Biopharmaceutic consideration of *in vitro* dissolution, *in vivo* pharmacokinetics, and deconvolution analysis indicated that *in vitro* dissolution was "too rapid" to adequately mimic *in vivo* dissolution. Findings suggest greater inspection of *in vitro* methods for poorly water-soluble drugs, especially those drugs where *in vivo* absorption is expected to be rate-limited by dissolution.

Keywords: IVIVC, Norvir® powder, dissolution, absorption, Wagner-Nelson

INTRODUCTION

In vitro-in vivo correlation (IVIVC) has various definitions.[1,2] We believe this reflects the frequent historical effort to relate *in vitro* dissolution with *in vivo* product performance, such that among the many potential uses of *in vitro* dissolution testing, one is to estimate or mimic *in vivo* dissolution. For example, "biorelevant media" are designed to be compositionally similar to *in vivo* gastrointestinal fluids, with the potential to then kinetically mimic *in vivo* dissolution. Interestingly, at least for immediate release (IR) oral solid dosage forms, it is uncommon to address "Is the *in vitro* dissolution profile mimicking the *in vivo* dissolution profile?". The question was investigated in this study using Norvir® oral powder. Containing 100 mg ritonavir (RTV) per packet, Norvir® oral powder was

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approved in 1996 in the United States and the European Union. RTV is a prototypical poorly water-soluble drug, requiring formulation as an amorphous solid dispersion (ASD).[3,4] The absolute oral bioavailability of each Norvir® oral powder and Norvir® tablet is about 70%.[5] Norvir® oral powder and Norvir® tablets use the same ASD intermediate, which involves hot melt extrusion of RTV and polyvinylpyrrolidone/vinyl acetate (PVPVA) as the polymer. Norvir® tablet and Norvir® oral powder are bioequivalent.[5] Although the tablet and oral powder are bioequivalent in C_{max} and area under the curve (AUC), they are not pharmaceutically equivalent and hence not therapeutically equivalent. The USP compendial dissolution method of Norvir® tablet employs 60 mM polyoxyethylene 10 lauryl ether (PE), a relatively high concentration of the surfactant. RTV drug substance solubility in 60 mM PE is 198 µg/mL, while it is only 2.338 µg/mL in media without 60 mM PE.[6] Since RTV is poorly water-soluble and its oral bioavailability is incomplete, we anticipated that RTN absorption is dissolution rate-limited.

In this study, the question pursued was "Is *in vitro* dissolution profile mimicking the *in vivo* dissolution profile?". Human *in vivo* data from Salem et al.[7] were used. Salem et al.[7] studied the pharmacokinetics of Norvir® oral powder under fasted, moderate fat, and high fat conditions. In our analysis here, absolute oral bioavailability was 70% for the fasting state, 54% for the moderate fat, and 47.8% for the high fat. The absolute oral bioavailability of each Norvir® oral powder and Norvir® tablets is about 70%.[5] From the Norvir® oral powder package insert, its oral bioavailability with a moderate fat meal is reduced 23% compared to fasted,[8] such that an absolute oral bioavailability was computed here to be 54%. Finally, according to Salem et al.,[7] the AUC ratio of moderate fat versus high fat is 1.13, such that an absolute oral bioavailability under high fat was computed here to be 47.8%. The aim of the study was to compare the *in vitro* dissolution profile to the *in vivo* dissolution profile of Norvir oral powder in fasted, moderate-fat fat and high-fat conditions.

MATERIALS AND METHODS

Materials

Packets of Norvir® oral powder (100 mg RTV per packet) (AbbVie; North Chicago, IL, USA) were commercially obtained. PE was purchased from Sigma Aldrich (St. Louis, MO, USA). The RTV active ingredient was from ChemShuttle (Blue Current Inc., Hayward, California). Solvents were of analytical grade and obtained from Fischer Scientific (Fischer Scientific; Hampton, NH) and Sigma-Aldrich (Sigma-Aldrich; St. Louis, MO).

In vitro dissolution profile of Norvir® oral powder

Dissolution testing was performed on Norvir® oral powder (containing 100 mg of RTV) in 900 mL of PE medium (50 mM maleic acid buffer with 60 mM polyoxyethylene 10 lauryl ether; pH 5.8) at 37 °C using 100 rpm with the USP-II apparatus (SR8PLUS, Hanson Research, Chatsworth, CA). Dissolution of a packet per vessel was performed in triplicate. 60 mM PE was used, since it is the USP compendial method for Norvir® tablets. A 2 mL sample was taken (at 0, 5, 10, 20, 30, 45, 60, 90, 120, 180, 240, and 360 min), and replaced with 2 mL of fresh PE medium at each time point. Then, samples were filtered through a 0.45 µm membrane filter and quantified using high-performance liquid chromatography (HPLC). The concentration of RTV was determined using an HPLC method.[6] Sample analysis was conducted with a Waters 2489 HPLC system (Waters Corporation, Milford, MA) equipped with an ultraviolet-visible detector (240 nm wavelength). An isocratic mobile phase comprising 47% of acetonitrile and 53% of 0.05 M phosphoric acid was employed, with an injection volume of 25 µL and a flow rate of 1 mL/min. Separation was achieved using a 4.6×150 mm Zorbax C18 column with a 5-µm particle size. RTV exhibited a retention time of 9–10 min, and the total run time was 13 min. A calibration curve with RTV concentrations of 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, 0.195, and 0.098 µg/mL was generated in triplicate for each analysis, yielding an R^2 value of 0.9999.

Application of mathematical models for drug dissolution kinetics

In vitro dissolution profiles were analyzed using regression analysis with five models: zero-order, first-order, Higuchi, Hixson-Crowell, and Korsmeyer-Peppas.[9-11] Norvir® oral powder dissolution was subjected to model fitting using DDSolver®. The correlation coefficient value (R^2) of each fit was obtained.[12] The zero-order equation is % dissolved = k_o*t, where k_o is the dissolution rate constant, and t is time. The first order equation is % dissolved = 100*(1 - e^{-k1t}), where k_1 is the first order dissolution rate constant.

Higuchi's equation is % dissolved = k_H *$t^{1/2}$}, where k_H is the Higuchi dissolution rate constant.

The Hixson-Crowell equation for percentage dissolved is: % dissolved = 100 * [1 - (1 – (k_{HC} * t / 4.6416))^3], where k_{HC} is the Hixson-Crowell release rate constant. The Korsmeyer-Peppas equation is % dissolved = k_{KP} * t^n, where k_{KP} is the Korsmeyer-Peppas release rate constant, and n is the release exponent.[13-16]

Wagner-Nelson deconvolution: in vivo dissolution profiles of Norvir® oral powder

In vivo human data of Norvir® oral powder under the fasted, moderate fat, and high fat conditions were extracted from the literature.[7] The data were digitized from the graphs using a plot digitizer. The deconvolution method was utilized to obtain *in vivo* drug absorption from the fasted, moderate-fat, and high-fat plasma concentration time profiles. Assuming *in vivo* drug absorption was limited by the *in vivo* dissolution rate, deconvolution would yield that the *in vivo* drug absorption profile is also the *in vivo* dissolution profile. The Wagner-Nelson deconvolution has been applied to RTV oral pharmacokinetic profiles previously[5], and was selected here as a deconvolution method, in part because it does not require intravenous data. The Wagner-Nelson equation is:

$$F_a = (C_p+(K_{el} * AUC_{0-t})) / (K_{el} *AUC_{0-\infty}) \quad \text{Equation 1}$$

Where F_a is the fraction of the dose absorbed at time t, C_p is the plasma concentration (ng/mL) of RTV at time t, K_{el} (1/hr) is the elimination rate constant, and AUC (ng*hr/mL) is the area

under the curve. K_{el} was calculated from the least-squares fitted terminal log-linear portion of the plasma concentration–time profile. K_{el} was calculated here to be 0.223/h, 0.354/h, and 0.246/h under fasted, moderate fat, and high fat conditions, respectively. AUC_{0-t} is the integration of concentration of RTV from time "0" to "t" (time of last quantifiable drug level), *i.e.* the AUC from time "0" to "t". $AUC_{0-\infty}$ is the integration of concentration of RTV from time '0' to '∞', *i.e.* area under curve from time '0' to 'infinity'.[17] Since it concerns the relative amount of drug absorbed at time t versus the final amount of drug that was absorbed, Wagner-Nelson profile analysis always yields 100% as a terminal value (*i.e.,* F_a = 1, or 100% of the amount that was absorbed). The percent absolute absorbed profile was also calculated as the percent absorbed profile (*i.e.*, Wagner-Nelson profile) and normalized by the absolute oral bioavailability, which is 70% for fasted, 54% for moderate fat, and 47.8% for high fat (see Introduction). The *in vitro* dissolution profile of Norvir® oral powder (from *in vitro* testing using PE media) was compared to *in vivo* dissolution profiles of Norvir® oral powder under fasted, moderate fat, and high fat conditions (from Wagner-Nelson analysis of pharmacokinetic profiles, normalized for extent of absorption).

Levy-Polli plot analysis

Levy-Polli plots involve the fraction of drug absorbed (F_a) versus the fraction of drug dissolved (F_d), and help to evaluate the relative contributions of drug dissolution and permeation to overall absorption kinetics.[18-21] Comparisons of F_a-F_d trajectory plots from *in vivo* PK data and from *in vitro* PE dissolution are typically conducted to assess underpinning kinetics of oral drug bioavailability, assuming the *in vitro* test profile mimics the *in vivo* dissolution profile. However, here, in light of the biopharmaceutical properties of RTV and Norvir® oral powder, it was assumed that *in vivo* drug absorption was limited by the dissolution rate, such that analysis focused on the ability of *in vitro* dissolution testing to mimic *in vivo* dissolution.

Scale factor analysis: polli equation and first-order equation

As the results show, the *in vitro* dissolution profile was much faster than the *in vivo* dissolution profile. Hence, to gauge the rate at which *in vitro* dissolution would need to be slowed to approximate *in vivo* dissolution, a scale factor was estimated. IVIVC analysis allows researchers to determine a scaling factor (SFs).[22] Two modeling approaches were taken to estimate an SF: the Polli equation and the first-order equation. For the Polli equation approach, the *in vitro* dissolution profile of Norvir® oral powder was fit to the Polli dissolution equation (Equation 2) to estimate the single fitted parameter *in vitro* k_d:[23]

$$\% \text{ dissolved} = 100 * [1 - ((M_0 - C_s*V)/(M_0 - C_s*V*e^{-kd((M0-Cs*V)/V)*t}))]$$

Equation 2

Where M_0 is the initial mass of drug in the dosage form, so it is the drug dose (100 mg), C_s is RTV solubility in PE media (0.198 mg/mL[6]), V is dissolution volume (900 mL), t is time (min), and k_d is the dissolution rate coefficient (mL/mg per min). The Polli equation is a simple, one-parameter (i.e., k_d) equation, and only requires regression. The equation can accommodate both sink and non-sink dissolution conditions. Equation 2 was fitted to % dissolved versus time data via Solver (Microsoft, Redmond, WA; version 2206) to estimate k_d.[23] Solver is a free Microsoft Excel add-in program from Microsoft and is intrinsic to Excel, although it may need to be initially loaded into Excel.[24] The initial estimate of k_d was 0.1 mL/mg per min. *In vivo* k_d values were also determined from the *in vivo* dissolution profiles of Norvir® oral powder for fasted, moderate fat, and high fat conditions (from Wagner-Nelson analysis of pharmacokinetic profiles). For all regressions, R^2 values of the fits were calculated.[10]

To summarize the rate at which *in vitro* dissolution would need to be slowed to approximate *in vivo* dissolution, an SF was estimated for each pharmacokinetic condition. For each fasted, moderate fat, and high fat condition, the SF value was taken to be the ratio of the *in vivo* k_d (from Wagner-Nelson analysis of pharmacokinetic profiles) over the *in vivo* k_d (from *in vitro* dissolution).[24] Additionally, the same analysis using the first-order equation was conducted to estimate an SF to compare *in vitro* and *in vivo* dissolution. As denoted above, the first-order equation [% dissolved = $100*(1- e^{-k1t})$] was used. Separately, it was applied to the *in vitro* dissolution profile and to each Wagner-Nelson profile (normalized for extent of absorption, under fasted, moderate fat, and high fat conditions).

Data analysis

The collected data were analyzed using SPSS Version 16 (Systat Software Inc., CA, USA). A t-test was used to compare two groups. Results were given as mean ± standard error of the mean (n=3). To compare the drug *in vivo* dissolution profiles to *in vitro* dissolution profiles, the f_2 calculation was conducted.[25]

RESULTS

In vitro dissolution profile of Norvir® oral powder

Dissolution of Norvir® oral powder in maleic acid buffer containing 60 mM polyoxyethylene10 lauryl ether (PE) (pH 5.8) is plotted in Figure 1. About 98% of RTV was dissolved in 5 min., and reached 100% in 4 hr (*i.e.*, 100 mg/900 mL or 0.111 mg/mL). Indulkar et al.[26] also observed fast *in vitro* release of RTV with PVPVA. Dissolution was conducted for 6 hours, with no evidence of drug precipitation. Because dissolution modeling can impart insight into dissolution mechanisms, Norvir® oral powder dissolution was subjected to model fitting. Among five models, the first-order and Korsmeyer-Peppas models best described RTV dissolution (R^2=0.9997 and R^2=0.9976, respectively). In the Korsmeyer-Peppas model, the value of the release exponent n characterizes the dissolution mechanism. Here, n=0.04 (*i.e.*, n≤0.45), reflecting Fickian diffusion (Case I diffusional).[27,28]

In vivo dissolution profiles of Norvir® oral powder

Figure 1 plots and Table 1 list the absolute fraction absorbed (F_a) from Wagner-Nelson analysis of human *in vivo* pharmacokinetic data, normalized for absolute oral bioavailability. The *in vitro* dissolution profile was also plotted. Of note, Wagner-Nelson

profile analysis alone always yields 100% as a terminal value (*i.e.*, F_a = 1, or 100% of the amount that was absorbed). Meanwhile, Figure 1 shows the percent absolute absorbed profile, represented by the Wagner-Nelson profile, which is normalized absolute oral bioavailability: 70% for fasted, 54% for moderate fat, and 47.8% for high fat. F_a in Figure 1 is taken to be equal to the *in vivo* dissolution profile, since RTV absorption from Norvir® oral powder is assumed to be rate-limited by *in vivo* dissolution, and not *in vivo* drug intestinal permeation or physiology such as gastric emptying.

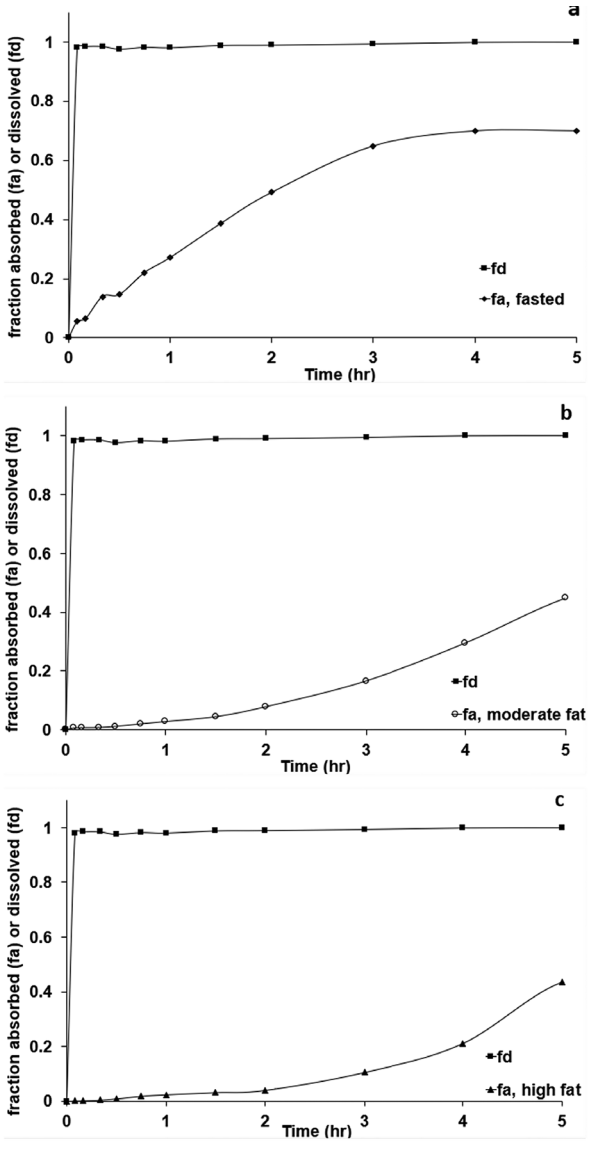

Figure 1. RTV fraction absolute absorbed (F_a) profiles from Wagner-Nelson analysis and fraction dissolved (F_d) from *in vitro* dissolution test.

*F_a was from Wagner-Nelson analysis of human *in vivo* pharmacokinetic data, under a) fasted, b) moderate fat, and c) high fat conditions.[7] F_a values are normalized for absolute oral bioavailability, which is 70% for the fasted state, 54% for a moderate-fat diet, and 47.8% for a high-fat diet. F_a is assumed to also be *in vivo* fraction dissolved, such that *in vitro* dissolution was much more rapid that *in vivo* dissolution under all conditions, including being "too rapid"

RTV: Ritonavir

In Figure 1, at 5 h, *in vitro* F_d was nearly 1 (*i.e.,* complete) while absolute F_a values were considerably lower, with 0.7 (Figure 1a for fasted), 0.447 (Figure 1b for moderate fat), and 0.436 (Figure 1c for high fat). Table 2 also lists F_d and F_a values. At 20 min, *in vitro* F_d was about 0.9998, while absolute F_a under fasted, moderate fat, and high fat conditions were only 0.140 (Figure 1a), 0.00737 (Figure 1b), and 0.00453 (Figure 1c), respectively. Pharmacokinetic inspection revealed a large difference between *in vitro* dissolution and *in vivo* dissolution. When comparing the fasted absorption profile, moderate fat absorption profile, and high fat absorption profile to the *in vitro* dissolution profile, f_2 was 8.34, 2.49, and 1.98. From Figure 1, F_a is assumed to also be the *in vivo* fraction dissolved, such that *in vitro* dissolution was much more rapid than *in vivo* dissolution under all conditions, including being "too rapid". The *in vitro* method contained a relatively high concentration of a pharmaceutical surfactant.

Levy-Polli plots of Norvir® oral powder and the implication of in vitro dissolution being "too rapid"

Levy-Polli plots are helpful to understand the relationship between F_a and F_d and to assess whether overall drug absorption is dissolution rate-limited, permeation rate-limited, or mixed dissolution/permeation rate-limited, assuming *in vitro* dissolution estimates *in vivo* dissolution. Figure 2 plots the relationship between F_d from *in vitro* dissolution in PE medium and *in vivo* absolute F_a under fasted (Figure 2a), moderate fat (Figure 2b), and high fat conditions (Figure 2c). Each Levy-Polli plot exhibited the reverse "L pattern" (Hockey-Stick trajectory) of Norvir® oral powder. While F_d values increased rapidly from 0 to over 0.9, *in vivo* F_a increased by only 0.055 for the fasted condition (Figure 2a), to 0.006 for moderate fat condition (Figure 2b), and to 0.001 for the high fat condition (Figure 2c). Also, at 5 hours, F_d was 0.99, while *in vivo* absolute F_a was 0.7 under the fasted state, 0.54 under the moderate fat, and 0.478 under the high fat conditions. Results point towards *in vitro* dissolution testing being "too rapid", at least if *in vitro* dissolution is intended to mimic *in vivo* dissolution. Hence, analysis proceeded to identify an SF to estimate the extent to which *in vitro* dissolution was too fast.

All plots exhibit a "reverse L" shape profile, reflecting the very rapid *in vitro* dissolution profile and not a "straight line" profile that would be expected from a dissolution-rate-limited absorption scenario. Results point towards *in vitro* dissolution testing being "too rapid", at least if *in vitro* dissolution is intended to mimic *in vivo* dissolution.

Scale factor analysis: polli equation and first-order equation

The Polli equation and the first-order equation were separately used to estimate a scale factor to summarize the degree that which the observed, rapid *in vitro* dissolution would need to be slowed, for the *in vitro* dissolution profile to mimic the *in vivo* dissolution profile. The Polli model was used to fit the *in vitro* dissolution (*e.g.,* Figure 1), as well as the *in vivo* dissolution profile (*e.g.,* each panel in Figure 1 for the three conditions). Fitting involved estimating the k_d value. For the fit to the *in vitro* dissolution profile in Figure 1, k_d was 7.15 mL/mg per min, a high value reflecting the rapid dissolution.

Table 1. Percent dissolved, percent absorbed and percent absolute absorbed values of RTV oral powder under fasted, moderate fat and high fat conditions

		Fasted		Moderate fat		High fat	
Time (min)	% dissolved	% absorbed	% absolute absorbed	% absorbed	% absolute absorbed	% absorbed	% absolute absorbed
0	0.0	0	0	0	0	0	0
5	98.1	7.8	5.5	1.1	0.6	0.3	0.1
10	98.5	9.1	6.4	1.2	0.7	0.4	0.2
20	98.5	19.9	13.9	1.4	0.7	0.9	0.5
30	97.6	20.8	14.6	2.1	1.1	1.9	0.9
45	98.2	31.5	22.1	3.7	2.0	3.9	1.9
60	98.1	38.7	27.1	5.1	2.8	5.0	2.4
90	98.8	55.2	38.7	8.2	4.4	6.7	3.2
120	99.0	70.2	49.2	14.5	7.8	8.4	4.0
180	99.4	92.7	64.9	30.6	16.5	22.1	10.6
240	99.8	100	70	54.5	29.5	44.2	21.1
360	100.0	100	70	100	54	100	47.8

*Dissolution was measured during *in vitro* testing. The percent absorbed profile was obtained from Wagner-Nelson analysis, yielding 100% as a terminal value (*i.e.,* 100% of the amount that was absorbed). The percent absolute absorbed refers to the percent absorbed profile normalized by absolute oral bioavailability, which is 70% for fasted, 54% for moderate fat, and 47.8% for high fat. RTV: Ritonavir

Table 2. Fitted Polli dissolution rate coefficient (k_d) and first order dissolution rate coefficient (k_1) values to *in vivo* fraction absolute absorbed profiles

In vivo dissolution	Fitted k_d (mL/mg per min) from the Polli equation	Scaling factor (unitless) from the Polli equation	Fitted k_1 (1/min) from the first-order equation	Scaling factor (unitless) from first-order equation
Fasted	0.0324	0.00453	0.009923	0.0127
Moderate fat	0.0078	0.00109	0.002968	0.00380
High fat	0.0058	0.000773	0.002586	0.00331

*Fraction absolute absorbed profiles are plotted in Figure 1 for fasted, moderate fat, and high fat conditions. Separate fits were performed for the Polli equation (k_d) and the first-order model (k_1). Separately, SFs were calculated for each Polli equation k_d and first-order k_1 for the *in vitro* fits of k_d and k_1 to be slowed down to match the *in vivo* fits of k_d and k_1. This *in vivo* analysis assumed dissolution-rate-limited absorption

Figure 3 compares fitted and observed profiles from *in vitro* dissolution, as well as the three *in vivo* conditions.

Fitted k_d values are shown in Table 2, along with fitted k_1 values. *In vivo* k_d values from fits to Figure 3 are listed in Table 2. K_d was 0.0324, 0.0078, and 0.0058 mL/mg per minute for *in vivo* F_a (*i.e., in vivo* dissolution) under fasted, moderate fat, and high fat conditions, respectively. Therefore, in comparing values, SFs were 0.00445, 0.001101, and 0.00842 for these conditions. Each scale factor was markedly less than one, indicating *in vitro* dissolution was many-fold "too rapid" (*e.g.,* about 200-fold for fasting condition), compared to *in vivo* dissolution.

A similar analysis based on fits to a first-order model provided comparable SFs and the same conclusion. k_1 was 0.782 min^{-1} for *in vitro* dissolution, indicating a notably high value reflecting rapid dissolution. *In vivo* k_1 was 0.009923, 0.002968, and 0.002586 min^{-1} for fasted, moderate fat, and high fat conditions, respectively. SF were 0.0127, 0.00380, and 0.00331 for these conditions. Again, each scale factor was markedly less than one, indicating *in vitro* dissolution was manyfold too rapid compared to *in vivo* dissolution. Regardless of whether the Polli equation or first-order model was used, the SF was always far less than unity (*i.e.*, less than 1), reflecting that the *in vivo* absolute absorption profile is much slower than the *in vitro* dissolution profile. Generally, SFs were about 0.01, reflecting that the *in vivo* drug absorption rate is 100-fold slower than *in vitro* dissolution. These SFs are qualitatively similar to the SF for a fast itraconazole ASD formulation, which was 0.0191.[24] Hence, there are ASDs with perhaps overly rapid *in vitro* dissolution testing, at least if *in vitro* dissolution is intended to mimic *in vivo* dissolution. Also, in Table 2, k_d values were about 2-4-fold larger than k_1 values. Correspondingly, the SF for k_1 was about 2-4-fold larger than the SF for k_d.

DISCUSSION

An alternative interpretation

An alternative interpretation is that the *in vitro* dissolution profile mimics the *in vivo* dissolution profile, such that *in vivo*

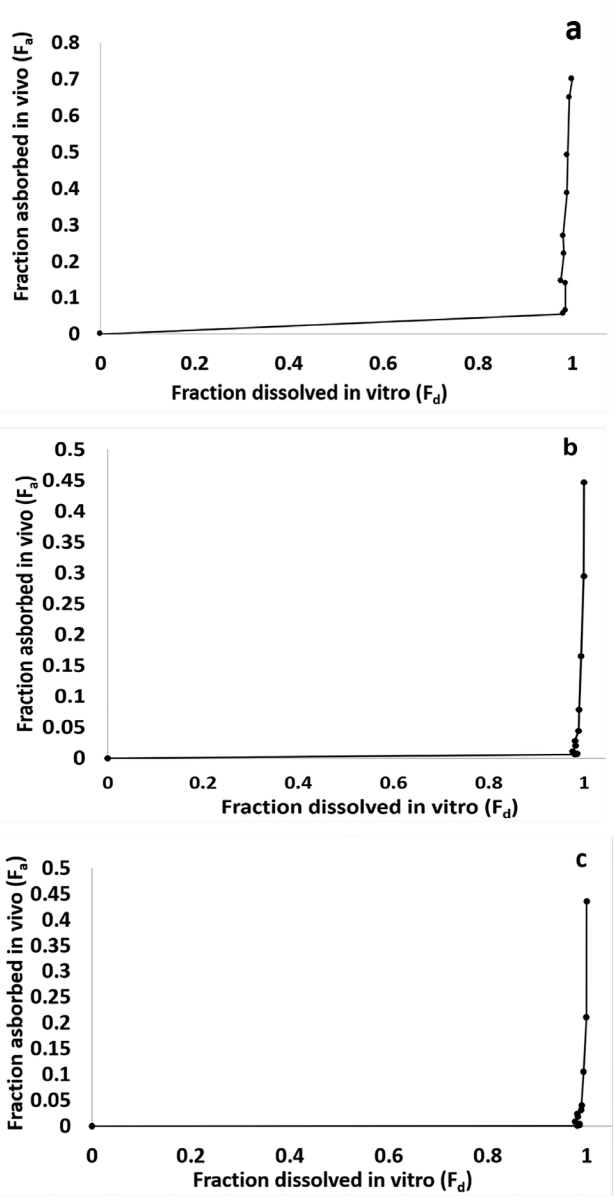

Figure 2. F_a vs. F_d relationships of Norvir® powder under a) fasted, b) moderate fat, and c) high fat conditions

dissolution is indeed nearly complete in 5-10 min, and that subsequent post-dissolution events (*e.g.*, drug permeation across the intestine) constitute the rate-limiting steps for overall RTN absorption. However, RTN has been reported to have a high intestinal permeability and be a Biopharmaceutics Classification System (BCS) Class 2 drug.[29] Of note, Karakucuk et al.[30] describe the challenges of measuring the permeability of the drug RTN with a very low solubility. Overall, however, we do accept an alternative interpretation that the very rapid *in vitro* RTN dissolution profile here mimics *in vivo* dissolution, and the incomplete and prolonged dissolution in the fasted state reflects low permeability. Some reports characterize RTN as BCS Class 2 or Class 4.[30-34] We believe the incomplete systemic availability of RTN (*i.e.*, 70%) could primarily reflect incomplete dissolution or partial (*e.g.,* 30%) first-pass metabolism. Incomplete absorption of a poorly soluble drug, can perhaps be preliminarily classed as BCS Class 4 (*i.e.,* a drug with low permeability), or it may simply reflect incomplete absorption of a Class 2 drug with very low solubility.

Comparison of scale factors

In a IVIVC study of itraconazole ASDs, a similar analysis was conducted. Itraconazole tablets were denoted to be Fast, Medium, and Slow dissolving, and this rank order was observed for both the *in vitro* dissolution and *in vivo*. However, *in vivo* dissolution for Fast, Medium, and Slow was only 0.0178 times, 0.213 times, and 0.217 times that of *in vitro* dissolution, respectively. That is, fast was about 50-fold slower *in vivo* than *in vitro*. Medium and Slow were each about 5-fold slower *in vivo* than *in vitro*.[24] Here, *in vitro* dissolution of Norvir® oral powder using a scale factor of 0.00453 based on k_d parameterization into a highly pharmaceutical surfactant was significantly more rapid, being about 200-fold faster than *in vivo* dissolution in the fasted state.

Evaluation of in vitro dissolution modeling to fit in vivo dissolution profiles

The Polli equation is a single-parameter dissolution equation, which means only the dissolution rate coefficient (k_d) is fitted in regressing the equation to the dissolution data, without the need for a fitted extent of dissolution parameter. The fitted k_d values were calculated to match those predicted by the Polli equation and the observed dissolution profiles. It was found to be 7.15 (mL/mg per min), which was a high value due to the IR profile. A similar fast release profile was observed in Itraconazole ASD, and k_d values were calculated as 30.12 (mL/mg per min).[24] Moreover, k_d values were calculated to fit the *in vitro* dissolution profiles to the *in vivo* absorption profiles. The units of k_d in Equation 2 were mL/mg per min, similar to those for the z-factor dissolution model.[23] After the comparison of the observed *in vitro* dissolution profiles of Norvir® powder with fitted *in vitro* dissolution profiles *via* the Polli equation, k_d values were calculated to align *in vitro* PE dissolution with *in vivo* dissolution in fasted, moderate fat, and high fat conditions. As shown in Table 2, the k_d values were calculated as 0.03, 0.008, and 0.006 mL/mg per min for fasted, moderate fat, and high fat conditions to match the dissolution profiles. While there was no significant difference (p=0.333>0.05) between the k_d values of the moderate fat and high fat conditions, the k_d value was lower than that of the fasted conditions. As perhaps expected, the dissolution rate coefficient, k_d was generally smaller when drug solubility (C_s) was larger.[23] It was confirmed by the literature.[35,36] Xu et al.[5] found that RTN solubility was 7.4±1.1 µg/mL in FaSSIF-V2 and 18.5±1.9 µg/mL in FeSSIF-V2. Kokott et al.[36] reported RTN solubility in FaSSIF was 5.4±0.6 µg/mL. Similar results were observed with the ketoconazole and itraconazole tablets. The k_d value of ibuprofen in FeSSIF-V2 (0.0780 mL/mg per min) was higher than ketoconazole in FaSSGF (0.0154 mL/mg per min)[23] due to the ibuprofen solubility in FeSSIF-V2 (1.76 mg/ mL) being lower than the solubility of ketoconazole in FaSSGF (11.2 mg/ mL).[37] Moreover, the reason for the lower k_d values of RTV in the fed state compared to the fasted state can be related to the

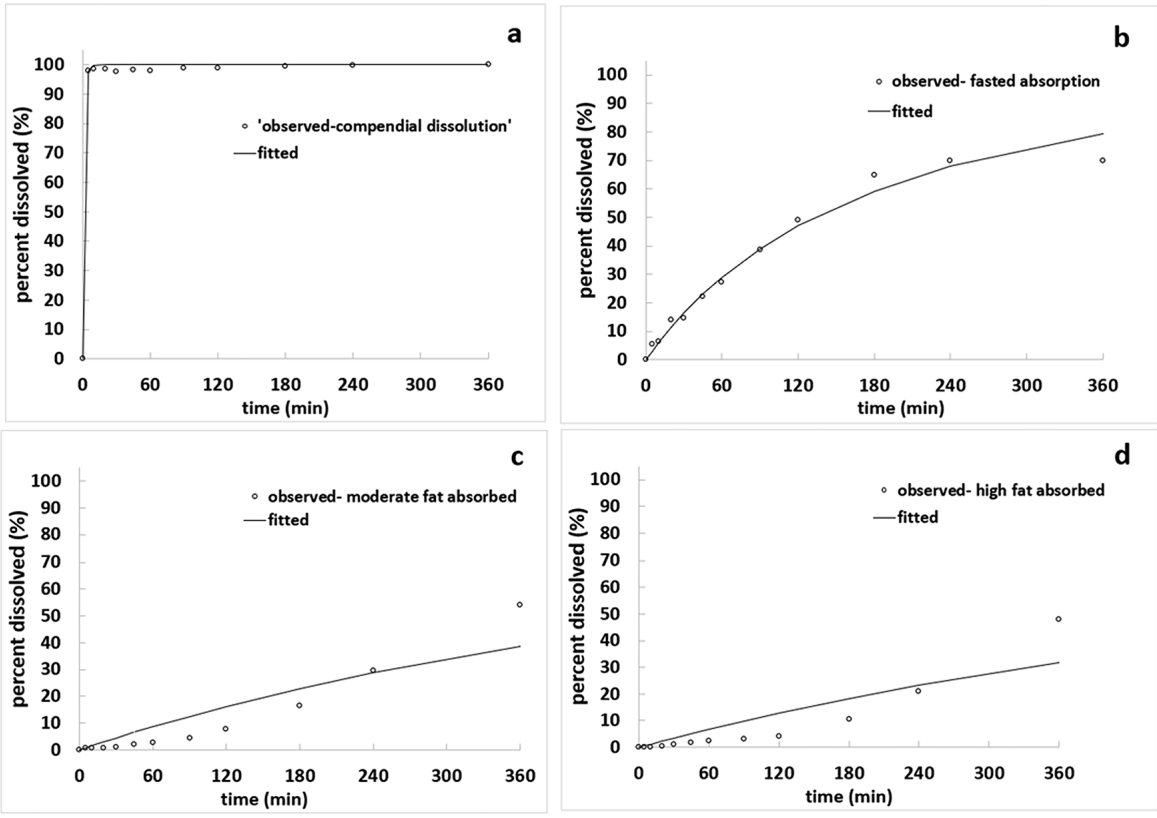

Figure 3. Fitted dissolution profiles *via* Polli equation and observed dissolution profiles for each food condition. Panels are *in vitro* dissolution in PE medium (a), and *in vivo* dissolution under fasted (b), moderate fat (c), and high fat (d) conditions

fact that the Fed state colloids are large and slowly diffusing relative to other biorelevant media, colloids.[38-40] In addition to the k_d values, S_f and first-order dissolution constants (k_1) were also calculated and given in Table 2. S_f is unitless and reflects a single algorithm for *in vitro* dissolution scaling. It is a multiplier with a value less than 1 that slows *in vivo* dissolution relative to *in vitro* dissolution. The k_d values from *in vivo* were 0.4% lower for the fasted condition, while they were 0.1% lower for the moderate fat and high fat conditions, compared to the k_d from the *in vitro* dissolution. These results were similar to the literature.[24] For example, *in vitro* dissolution of itraconazole was faster than its *in vivo* dissolution, similar to the results obtained here. Moreover, the k_1 values were calculated from the first-order dissolution equation, which is a common differential equation for fitting percent dissolved versus time profiles. Compared to the Polli equation, the first-order equation, [% dissolved = $100*(1 - e^{-k1t})$], has a limitation due to its solution not accommodating a solubility limit impact on the percentage dissolved. As it is shown in Table 2, the rank order of the k_1 values was similar to the k_d values. The k_1 values under the fasted state were higher than those under the fed states due to the presence of high solubility and slow diffusion of large colloids under the fed conditions.[23]

CONCLUSION

The present investigation performed *in vitro* dissolution of Norvir® oral powder and Wagner-Nelson deconvolution of *in vivo* data to elucidate the relevance of *in vitro* dissolution testing. Qualitatively, there was a large difference between *in vitro* dissolution and *in vivo* dissolution. *In vitro* dissolution showed 98% release in 5 min. Meanwhile, from Wagner-Nelson analysis, only 5.5% of the drug dissolved (and was absorbed) *in vivo* in 5 min under fasted conditions. 49% of the RTV dose dissolves (and is absorbed) *in vivo* after 2 hours. It was concluded that such rapid *in vitro* dissolution was not mimicking *in vivo* dissolution of this poorly water-soluble drug, as it has been reported to have high intestinal permeability. Rather, *in vitro* dissolution, which involved a high surfactant concentration, was "too rapid,", while *in vivo* dissolution was better estimated by Wagner-Nelson analysis. For each pharmacokinetic condition (*i.e.,* fast, moderate fat, and high fat), a scale factor was estimated to approximate the degree to which *in vitro* dissolution needed to be slowed to mimic *in vivo* dissolution. For fasting, *in vitro* dissolution needed to be slowed by about 100-fold. Findings suggest greater inspection of *in vitro* methods of poorly water-soluble drugs, or at least those drugs where *in vivo* absorption is expected to be rate-limited by *in vivo* dissolution.

Ethics

Ethics Committee Approval: Not required.

Informed Consent: Not required.

Footnotes

Authorship Contributions

Concept: A.N.O., J.E.P., Design: A.N.O., Data Collection or Processing: A.N.O., Analysis or Interpretation: A.N.O., J.E.P., Literature Search: A.N.O., J.E.P., Writing: A.N.O., J.E.P.

Conflict of Interest: The authors declare no conflicts of interest.

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ORIGINAL ARTICLE

Design, Development and Evaluation of Orally Disintegrating Mini-tablets (ODMTs) Containing Cefixime for Paediatric Use: A Novel Approach

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ABSTRACT

Objectives: The study aimed to combine instant-release and mini-tablet methodologies to develop novel orally disintegrating mini-tablets (ODMTs) for a frequently pescribed antibiotic, cefixime trihydrate (CT), in paediatric patients.

Materials and Methods: CT-loaded microcapsules were prepared using Eudragit® EPO and Hydroxy Propyl Methyl Cellulose E50 by spray drying technique. The optimized microcapsules were mixed with co-processed ready-to-use tableting excipients, Ludiflash and Pearlitol 200SD, in different proportions and then compressed into ODMTs and evaluated.

Results: The particle size of CT microcapsules was found to be between 111.8 and 225.87 µm, suitable for oral delivery. The entrapment efficiency was found to be between 94.8% and 95.45%. Pre-compression and post-compression parameters indicated suitability for formulation. *In vitro* evaluation of ODMTs showed immediate disintegration within 15 seconds in the oral cavity as soon as they came in contact with saliva. Upon swallowing these microcapsules, the microcapsules completely dissolve in the gastrointestinal tract, releasing 100% of the drug (CT) within 15 minutes. The release kinetics of ODMTs were found to follow Hixson-Crowell kinetics for Eudragit® EPO and Korsmeyer-Peppas kinetics for Hydroxy Propyl Methyl Cellulose E50 microcapsules. Scanning electron microscopy images demonstrated that the microcapsules were intact even after compression into ODMTs. Stability studies proved that the formulations were stable as per the International Council for Harmonisation guidelines.

Conclusion: A novel solid oral dosage form of CT ODMTs of 2 mm diameter was successfully developed using Eudragit® EPO and Hydroxy Propyl Methyl Cellulose E50 in combination with co-processed ready-to-use tableting excipients such as Ludiflash and Pearlitol 200SD.

Keywords: Cefixime, microcapsules, mini tablets, instant release, paediatrics

INTRODUCTION

Orally disintegrating mini-tablets (ODMTs) are innovative solid oral dosage systems that quickly disintegrate in the oral cavity when they come in contact with saliva, typically within 10 to 15 seconds, without the requirement of water. ODMTs are more appropriate for paediatrics as they are tiny, have an agreeable mouthfeel, and fast disintegration in the mouth.[1]

Powders, multiparticulates, and/or dispersible formulations were discussed to be suitable for 6 months to 2 years of age.[2] High temperature frequently causes stability problems for liquid preparations. High costs of transportation and storage are considered. Hence, fluids should be avoided whenever possible.[3] At the age of 6 months, the child can physiologically and structurally gulp multiparticulates in a soft diet and

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brews, depending on the dimensions, contour, and solidity of the particles.[4] Child-appropriateness of the doses is specified by stress-free administration, palatability, the option for weight-based medication, and the use of safe, entrenched, and established excipients.[5] Hence, expertise in flexible solid platforms is essential. Hence, the planning of mini-tablets is escalating in the paediatrics field as required by European regulatory necessities for paediatric use.[6]

The Food and Drug Administration of the USA permitted Cefixime trihydrate (CT) in April 1989. Similar to other cephalosporins, this antibiotic halts bacteria from proliferating by preventing the formation of cell walls. The walls are essential to protect bacteria from the environment and to retain the contents. It shows a 3-hour elimination half-life with twice daily intake or, in some cases, once daily intake. Relative trials show that the medical and microbiological effectiveness of CT is more effective compared to co-trimoxazole (trimethoprim + sulphamethoxazole) or amoxycillin/clavulanic acid, and also more effective than cefaclor and cefroxidine in cases of acute lower respiratory tract infections and acute tonsillitis or pharyngitis. CT is resistant to hydrolysis by variants of wide-spectrum β-lactamases, and its stability is superior to cephalexin, cephradine, and cefadroxil. It was hence planned to prepare CT ODMTs.[7]

In the present research, our formulated ODMTs have two portions, namely microcapsules and minitablets. The microcapsule portion contains a drug encapsulated within Eudragit® EPO (EPO) and Hydroxy Propyl Methyl Cellulose E50 (HPMC E50) polymer coatings, which exclusively disintegrate in gastric pH (<5) and are insoluble in the salivary pH of 7. Then minitablets are formulated by taking optimized microcapsules and mixing them with ready-to-use, co-processed tableting excipients, namely ludiflash and pearlitol, which are also super disintegrants that disintegrate in the oral cavity upon contact with saliva (pH about 7), typically within a few seconds. Therefore, this study aimed to investigate the suitability of ready-to-use tabletting excipients ludiflash and pearlitol, in formulating ODMTs, and also to examine the taste masking as well as controlled release ability of EPO and HPMC E50 polymers in formulating microcapsules based on the various parameters tested.

MATERIALS AND METHODS

Materials

The drug CT was obtained as a gift sample from Karnataka Antibiotics Pvt. Ltd. (Bangalore, Karnataka). The ready-to-use tablet excipients Ludiflash and Pearlitol 200SD were received as a gift sample from Evonik Industries (Mumbai, Maharashtra), the lubricants Sodium Stearyl Fumarate (SSF) and Magnesium Stearate were purchased from Loba Chemie Pvt. Ltd. (Mumbai, Maharashtra). Polymers EPO and HPMC E50 were purchased from Rolex Chemical Industries (Bangalore, Karnataka). Solvents dichloromethane (DCM) and ethanol were purchased from Welchem Chemicals (Bangalore, Karnataka), cherry flavour was purchased from Keva Flavours (Mumbai, Maharashtra), and tablet compression facilities were availed at Apotex Research Pvt. Ltd. (Bangalore, Karnataka).

Methods

Preformulation spectroscopic studies

Determination of absorption maxima (λ_{max}) and standard curve of CT in methanol, 0.1 N HCl, and phosphate buffer 6.8

A stock solution (1000 µg/mL) of CT was made separately in methanol, 0.1 N HCl, and phosphate buffer 6.8. The sample was suitably diluted to get a concentration of 10 µg/mL using respective solvents and analyzed in the range of 200 nm to 400 nm using a Shimadzu ultraviolet visible (UV) spectrophotometer UV-1800, and λ_{max} was determined against a reagent blank. The experimentation was done in triplicate, and a standard curve was generated and plotted for the Beer-Lambert range.

Drug-excipient compatibility studies

In the current work, the potassium bromide pellet technique was used to check the compatibility of excipients with drug CT by taking their Fourier transform infrared spectroscopy (FTIR) spectra using Shimadzu FTIR 84900.[8]

Preparation of taste-masked microcapsules of CT

A total of 10 microcapsule formulations were prepared by varying proportions of drug CT and polymer (EPO and HPMC E50) in 1:1 to 1:5 ratios, using the spray drying method as shown in Table 1. The weighed quantity (as shown in Table 1) of polymers EPO and HPMC E50 was dispersed in the required quantity of DCM and ethanol in a 1:1 ratio.[9] The mixture was stirred for 30 minutes to achieve complete dispersion of polymers. To this, weighed quantities of CT and magnesium stearate (used as a glidant) were added and stirred on a magnetic stirrer for another 30 minutes to ensure uniform dispersion of the drug. The investigational considerations of the spray drying procedure were: outlet temperature 400 °C, inlet temperature 500 °C, aspirator setting 40, feed pump rate 2.5 mL/min, and 0.5 mm spray nozzle.[10] The formulation of various CT microcapsules is shown in Table 1.

Evaluation and characterization of the prepared CT microcapsules

Physical appearance of CT microcapsules

Visual examinations of the prepared CT microcapsules were performed.

Theoretical yield of CT microcapsules

The theoretical yield was measured based on the quantity of solid added to formulate a solution.

Practical yield of CT microcapsules

Formulated CT microcapsules were recovered from the spray dryer and then weighed.

Table 1. Formulations chart of CT microcapsules

Formulation code	CT (gms)	EPO (gms)	HPMC E50 (gms)	Magnesium stearate (*w/w*%)	1:1 of DCM and ethanol
F1	5	5	-	1	q. s. to prepare a solution of 10%
F2	5	10	-	1	q. s. to prepare a solution of 10%
F3	5	15	-	1	q. s. to prepare a solution of 10%
F4	5	20	-	1	q. s. to prepare a solution of 10%
F5	5	25	-	1	q. s. to prepare a solution of 10%
F6	5	-	5	1	q. s. to prepare a solution of 10%
F7	5	-	10	1	q. s. to prepare a solution of 10%
F8	5	-	15	1	q. s. to prepare a solution of 10%
F9	5	-	20	1	q. s. to prepare a solution of 10%
F10	5	-	25	1	q. s. to prepare a solution of 10%

DCM: Dichloromethane, gms: Grams, HPMC: Hydroxypropyl methylcellulose, q. s.: Quantity sufficient

Percentage yield of CT microcapsules

The percentage yield was measured using the Equation 1:

$$\%\, yield = \frac{\text{practical mass (microcapsules)}}{\text{theoretical mass (drug + polymer)}} \times 100 \quad \text{(Equation 1)}$$

Encapsulation and entrapment efficiency of CT microcapsules

CT microcapsules were crushed in a glass mortar and pestle; the powder equivalent to 50 mg of CT was dissolved in 100 mL of methanol and sonicated for 5 minutes; filtered, and 1 mL of filtrate was pipetted out and diluted to 100 mL of methanol. One mL from this solution was pipetted out and made up to 10 mL using methanol. The drug content was determined using a Shimadzu spectrophotometer (UV-1800) against a reagent blank with a maximum absorbance at 290 nm.[11] The drug encapsulation effectiveness was calculated by the following equation:

$$\%\, encapsulation\, efficiency = \frac{\text{Practical Yield}}{\text{Theoretical Yield}} \times 100 \quad \text{(Equation 2)}$$

Particle size examination of CT microcapsules

Particle size analysis of approximately 300 randomly selected CT microcapsules was conducted using optical microscopy.[12]

Surface morphology of CT microcapsules by scanning electron microscopy (SEM)

The CT microcapsules were prepared by mounting the sample on the sample-holder made with a solid Aluminium stage (Al slab) using carbon (C) adhesive tape. Then, samples are degassed for 72 hours in a desiccator. Then, 10 nm gold (Au) depositions for conductivity will be applied using Quorum Technology equipment. The samples are then loaded into the SEM machine (Ultra 55 FESEM from Carl Zeiss), and after achieving a high vacuum, SEM images of optimised microcapsule formulations were taken.[13]

In vitro dissolution of CT microcapsules

In vitro release of CT microcapsules was done using the United States Pharmacopoeia (USP) type II dissolution apparatus (paddle) (Electro lab TDT-08L). Using 900 mL of 0.1 N HCl as dissolution media, the apparatus was set at 50 rpm for 30 minutes and 37±0.5 °C temperature. At predetermined intervals (0, 5, 10, 15, 20, 25, 30 minutes), 5 mL of the samples were taken out and substituted with 5 mL of the fresh dissolution media. The samples withdrawn were suitably diluted, and the concentration of CT was measured using a UV spectrophotometer against a reagent blank.[14] The same procedure was performed by taking 900 mL of phosphate buffer, pH 6.8, as a dissolution medium.

Drug release kinetics of CT microcapsules

Based on the above evaluation parameters, selected CT microcapsule formulations were fitted to different kinetic models like zero-order, 1st order, Higuchi, Korsmeyer-Peppa's, and Hixson-Crowell cube root law using the software BCP soft to predict the drug release mechanism.[15]

Stability studies of CT microcapsules

Optimised CT microcapsule formulations were kept in an air-tight container and packed in aluminium foil, and sealed tightly. It was stored at temperatures and RH of 5 °C/ambient, 25°±2°C and 60±5% RH, 40°±2°C, and 75±5% RH, and evaluated at the time intervals of 30, 60, and 90 days as per ICH guidelines.[16]

Formulation of ODMTs

ODMTs of CT were prepared using a rotary tablet press in the direct compression process with multi-tip punches.[17] The optimised CT microcapsules were mixed with ready-to-use co-processed tableting excipients, ludiflash and pearlitol 200SD, in different proportions (1:1, 2:1 and 3:1) and blended with SSF in 3.5% *w/w* (as lubricant and anti-adherent) and cherry flavour. A compression force ranging from 8 kN to 10 kN was applied to compress biconvex mini tablets with a diameter of 2 mm and weighing approximately 12 mg each.[18] Various ODMTs formulations were shown in Table 2.

Evaluation of formulated ODMTs for pre-compression parameters[19]

Bulk density (D_b) of the ODMTs blend

It was calculated as the ratio of the entire mass of powder to its bulk volume. The evaluation was conducted by taking a known quantity of powder into a measuring cylinder, and its volume was noted. It was measured in gm/mL and was calculated by.

$$D_b = \frac{M}{V_b} \quad \text{(Equation 3)}$$

M = powder mass, V_b= powder bulk volume.

Tapped density (D_t) of the ODMTs blend.

It was calculated by the ratio of the entire mass of powder and its tapped volume. It was evaluated by tapping the powder to a constant volume and was measured in gm/mL, and was calculated by,

$$D_t = \frac{M}{V_t} \quad \text{(Equation 4)}$$

M = powder mass, V_t = powder tapped volume.

Angle of repose (θ) of ODMTs blend

It is a measure of the frictional forces within loose powder. The powder was directed to flow into a funnel fixed on a stand at a definite height. Then, θ was measured by taking the height of the pile of powder and its radius. It was measured in degrees and was calculated by,

$$\tan\theta = \frac{h}{r} \quad \text{(Equation 5)}$$

h = height, r = radius.

Carr's index (I) of ODMTs blend

It gives the ease of flow of the material, expressed as a percentage. It was calculated by,

$$I = \frac{D_t - D_b}{D_t} \times 100 \quad \text{(Equation 6)}$$

D_t= powder tapped density, D_b= powder bulk density.

Hausner's ratio of the ODMTs blend

It is an indirect measure of the ease of flow of powder and was measured by,

$$\text{Hausner's Ratio} = \frac{D_t}{D_b} \quad \text{(Equation 7)}$$

D_t= powder tapped density, D_b= powder bulk volume.

Evaluation of formulated ODMTs for post-compression parameters[20]

Physical appearance of ODMTs

Physical appearances of ODMTs were inspected visually.

Crushing strength of ODMTs

The ODMT's resistance to crushing was tested by the Dr. Schleuniger Pharmatron (USA) crushing tester and expressed in Kp or newtons.

Physical parameters of ODMTs

The diameter and thickness of the ODMTs were measured using a Vernier calliper, Digimatic caliper, Japan, and expressed in mm.

Friability test (F) of ODMTs

The *F* test was conducted to confirm the strength of the tablets to resist mechanical shocks, which may occur during preparation. The friability of the tablets in all the formulations

Table 2. Various ODMT formulations with ready-to-use co-processed tableting excipients ludiflash and pearlitol 200SD

Formulation code	Optimised EPO microcapsule powder (gms)	Optimised HPMC E50 microcapsule powder (gms)	Ludiflash (gms)	Pearlitol 200SD (gms)	SSF (*w/w*%)	Cherry flavour
FF1	10	-	9.3	-	3.5	q. s.
FF2	10	-	-	9.3	3.5	q. s.
FF3	10	-	4.5	-	3.5	q. s.
FF4	10	-	-	4.5	3.5	q. s.
FF5	10	-	2.9	-	3.5	q. s.
FF6	10	-	-	2.9	3.5	q. s.
FF7	-	10	9.3	-	3.5	q. s.
FF8	-	10	-	9.3	3.5	q. s.
FF9	-	10	4.5	-	3.5	q. s.
FF10	-	10	-	4.5	3.5	q. s.
FF11	-	10	2.9	-	3.5	q. s.
FF12	-	10	-	2.9	3.5	q. s.

EPO: Eudragit® EPO, gms: Grams, HPMC: Hydroxypropyl methylcellulose, ODMT: Orally disintegrating mini-tablet, q. s.: Quantity sufficient to 0.35 mL, SSF: Sodium stearyl fumarate

was determined using a friabilator (Electrolab, Mumbai, India). It was rotated for 100 revolutions, and the % friability was calculated by employing the equation:

$$F(\%) = \frac{W_{initial} - W_{final}}{W_{initial}} \times 100 \quad \text{(Equation 8)}$$

The pharmacopoeial limit for F was not more than 1%.

Uniformity of the mass (weight variation) of ODMTs

For the test, 20 ODMTs were individually weighed, the average weight was calculated, and the % deviation was calculated using the equation,

$$\%\,deviation = \frac{|W_i - \bar{W}|}{\bar{W}} \times 100 \quad \text{(Equation 9)}$$

The pharmacopoeial limit was ±10%, and only if not more than two tablets fall outside the prescribed limits will the tablets pass the test.

Simulated wetting test (SWT) of ODMTs

SWT was the time taken for complete wetting of ODMTs using simulated salivary fluid to mimic the physiological environment of a wet tongue (*i.e.,* pH of saliva ranges from 6.2 to 7.6). It was carried out by placing a tablet on a piece of double-folded tissue paper in a Petri-plate containing 6 mL of simulated salivary fluid at pH 6.8 (phosphate buffer pH 6.8), and the time taken for the complete wetting of the upper plane of the tablet was noted. The SWT time of 10 randomly selected ODMTs in each batch was analysed.

In vitro dispersion test of ODMTs

In vitro dispersion time of ODMTs was analysed by keeping an ODMT in a beaker that contained 5 mL of distilled water, and the time necessary for complete dispersion was noted. The *in vitro* dispersion time of 10 randomly selected ODMTs in each batch was analysed.

Drug content of ODMTs

ODMTs were crushed and powder equivalent to 50 mg of CT was dissolved in 0.1 N methanol, filtered through Whatman filter paper (number 41), suitably diluted with 0.1 N methanol, and then the drug content was analysed by UV spectrophotometer against a blank.

In vitro disintegration test (DT) of ODMTs

The *in vitro* DT of ODMTs was analysed using a tablet disintegrator tester. Each of the six ODMTs was individually placed in a tube of the disintegrator, and then the discs were placed on them. The DT was carried out using distilled water as the medium with a temperature of 37±1 °C. The pharmacopoeial disintegration limit for tablets should not be more than 60 seconds.

In vitro dissolution studies of ODMTs

The test was performed by taking ODMTs equivalent to 50 mg of CT, which were first dispersed in phosphate buffer pH 6.8 to release the microcapsules. These microcapsules were then subjected to an *in vitro* dissolution test using a USP type II dissolution apparatus (paddle), utilizing 900 mL of 0.1 N HCl as the dissolution medium. The temperature of the water bath was maintained at 37.0±0.5 °C, and paddle rotation was set at 50 rpm. The sample (5 mL) was withdrawn at time intervals of 0, 5, 10, and 15 minutes and replaced with the same quantity of fresh dissolution media. The collected samples were suitably diluted with 0.1 N HCl and were analyzed using a UV spectrophotometer against a blank.

Drug release kinetics of ODMTs

In vitro drug release results of ODMTs were fitted to different kinetic models, namely zero-order, first-order, Higuchi, Korsmeyer-Peppas, and Hixson-Crowell cube root law, using the software BCP Soft, to analyze the drug release pattern from ODMTs.

SEM of ODMTs

SEM studies were performed for selected final formulations of ODMTs, similar to SEM studies of microcapsule formulations as explained earlier.

Stability of ODMTs[21,22]

A stability study of ODMTs was carried out, similar to stability studies of microcapsules as explained earlier.

Statistical analysis

The data obtained for different formulations was analyzed by one way analysis of variance.

RESULTS

Preformulation spectroscopic studies

Determination of λ_{max} and standard calibration curve of CT

Absorption maxima of CT were found to be 290.0 nm in methanol, 285.0 nm in 0.1 N HCl, and 288.0 nm in phosphate buffer at pH 6.8. The linearity was established using the Beer-Lambert law in the concentration range of 2 to 16 µg/mL.

Drug-excipient compatibility studies

The FTIR spectral studies showed primary peaks at wavelengths of 3566, 3531 cm^{-1} (N-H stretching), 3295 cm^{-1} (symmetric and asymmetric N-H stretching), 2946 cm^{-1} (C-H stretching), 1772 cm^{-1} (C=O stretching), 1591 cm^{-1} (C=N stretching), 1063 cm^{-1} (C-O stretching), and 1025 cm^{-1} (N-O stretching). The principal peaks of the CT combinations with polymers (Eudragit EPO and HPMC E50) and excipients (Ludiflash and Pearlitol 200SD) showed no shift or loss of characteristic peaks, indicating no interaction between drug CT, polymers, and excipients, thereby proving the drug-excipient compatibility Figures 1-5.

Evaluation and characterization of the prepared CT microcapsules[22]

Physical appearance of CT microcapsules

The obtained EPO and HPMC E50 microcapsules were found to be free-flowing and white.

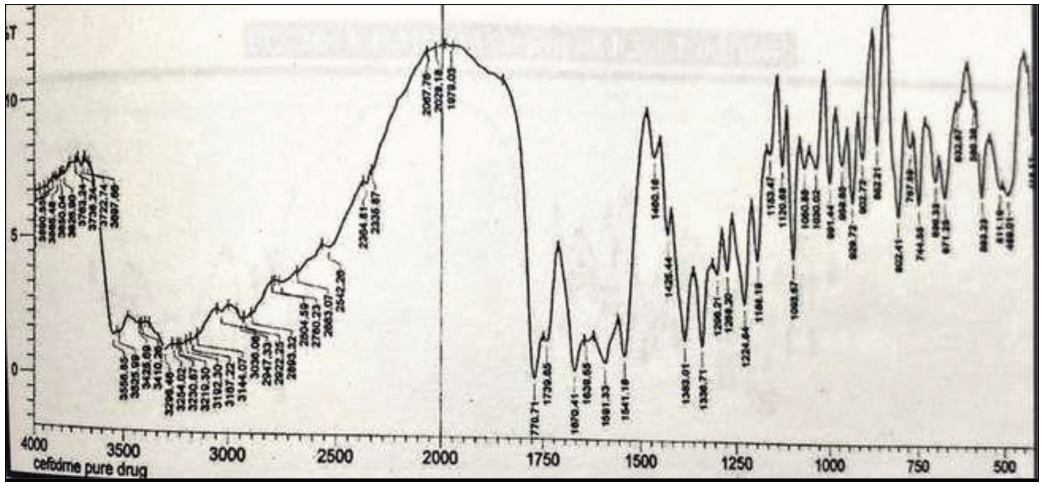

Figure 1. FTIR of the CT working reference standard

CT: Computed tomography, FTIR: Fourier transform infrared spectroscopy

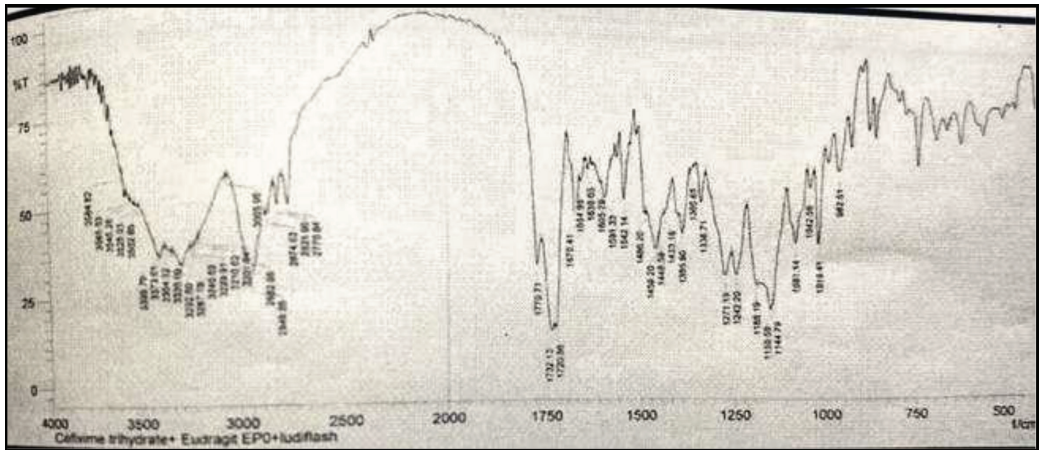

Figure 2. FTIR of FF5 ODMTs (CT + optimised EPO microcapsules + ludiflash)

CT: Computed tomography, EPO: Eudragit® EPO, FTIR: Fourier transform infrared spectroscopy, ODMTs: Orally disintegrating mini-tablets

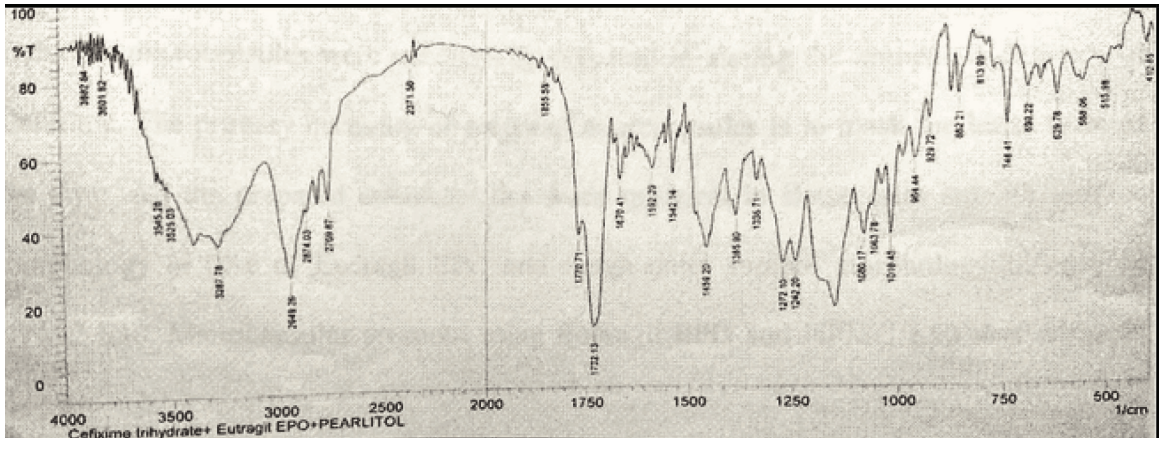

Figure 3. FTIR of FF6 ODMTs (CT + optimised EPO microcapsules + pearlitol)

CT: Computed tomography, EPO: Eudragit® EPO, FTIR: Fourier transform infrared spectroscopy, ODMTs: Orally disintegrating mini-tablets

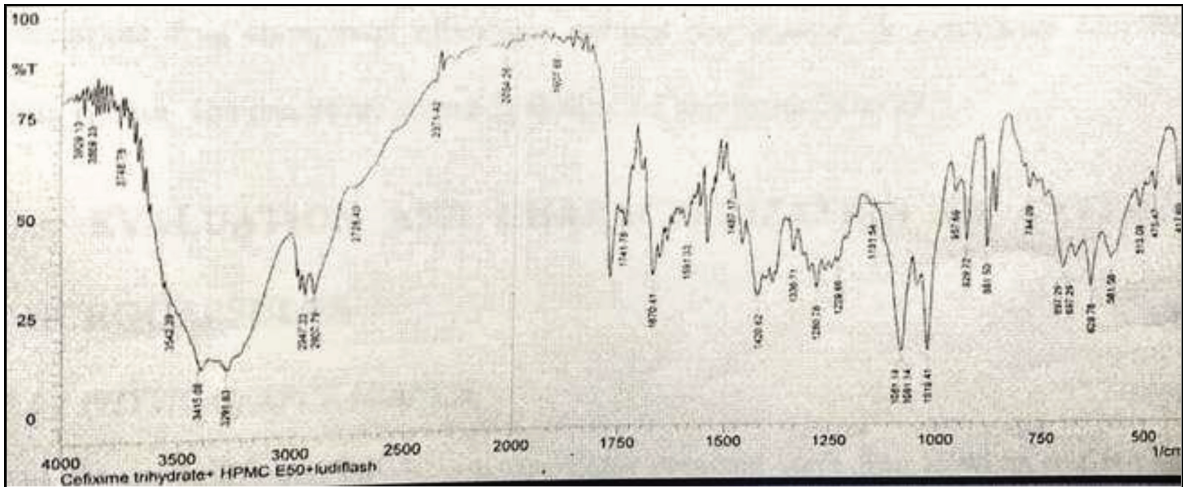

Figure 4. FTIR of FF11 ODMTs (CT + optimised hydroxypropyl methylcellulose E50 microcapsules + ludiflash)
CT: Computed tomography, EPO: Eudragit® EPO, FTIR: Fourier transform infrared spectroscopy, ODMTs: Orally disintegrating mini-tablets

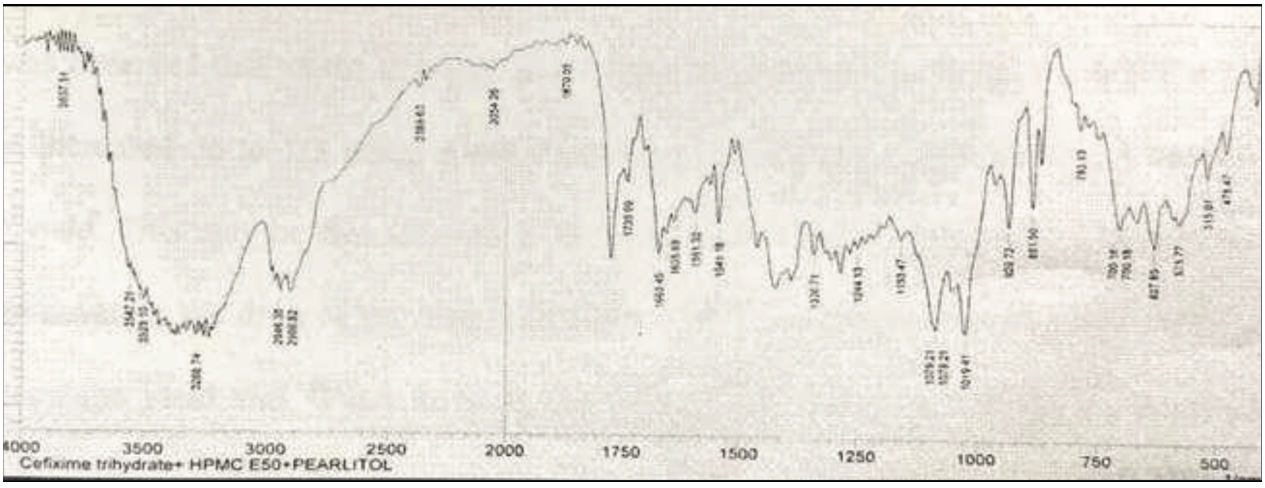

Figure 5. FTIR of FF12 ODMTs (CT + optimised hydroxypropyl methylcellulose E50 microcapsules + pearlitol)
CT: Computed tomography, EPO: Eudragit® EPO, FTIR: Fourier transform infrared spectroscopy, ODMTs: Orally disintegrating mini-tablets

Theoretical yield, practical yield, percentage yield, and encapsulation efficiency of CT microcapsules

The practical yield was compared with that of the theoretical yield, and the percentage yield was found to be in the range of 83.5% to 92.5%. It was observed that as the polymer ratio in the CT Eudragit EPO and HPMC E50 microcapsule formulation increased, the % yield, drug content, and entrapment efficiency also increased up to a 1:3 ratio of drug and polymer. A further increase in polymer concentration has decreased the yield. Thus, the optimized Eudragit EPO and HPMC E50 microcapsule formulation was found to be F3 and F8, respectively. These results were shown in Table 3.

SEM of CT microcapsules

The results of SEM of the optimised batch of CT Eudragit EPO and HPMC E50 microcapsules showed that they were spherical, having a smooth outer plane and particle size 125.45± 6.15 µm in case of CT Eudragit EPO microcapsules (F3) and rough outer surface and particle size 200.20±8.94 µm in case of CT HPMC microcapsules (F8) Figures 6, 7.

In vitro dissolution of CT microcapsules

The *in vitro* dissolution of CT Eudragit EPO and HPMC E50 microcapsules revealed that there was no drug release at phosphate buffer pH 6.8 (salivary pH) and 100% cumulative drug release (CDR) at a pH of 1 in 0.1 N HCl (gastric pH) within 15 minutes for optimised formulations F3 and F8. It was proven that microcapsules remained intact in the oral cavity and dissolved only in the stomach, releasing the complete drug within 15 minutes, as shown in Table 4.

Drug release kinetics of CT microcapsules

The linearity regression coefficient (R^2) values of the Hixson-Crowell cube root equation for CT-loaded Eudragit EPO microcapsules were found to be 0.9992, and the plot revealed linearity. The R^2 values of the Korsmeyer-Peppas model equation for CT-loaded HPMC E50 microcapsules were found to be 0.9997, and the plot revealed linearity. From these models, it was evident that at the initial stages, it might follow Hixson-Crowell release and Korsmeyer-Peppas pattern due to the small size of the microcapsules, which maintained

Table 3. Various evaluation parameters of CT microcapsules

Formulation	Physical appearance	Average particle size (µm) (Mean ± SD)	Yield %	Drug content (mg)	Drug entrapment Efficiency %
F1	Free-flowing and white	111.80±4.67	89.00	38.5	77.19
F2	Free-flowing and white	117.97±18.23	86.33	42.10	84.20
F3	Free-flowing and white	125.45±6.15	92.50	47.36	94.8
F4	Free-flowing and white	131.30±13.08	88.00	45.61	91.22
F5	Free-flowing and white	145.60±16.15	83.66	43.83	87.66
F6	Free-flowing and white	120.90±15.58	90.00	39.64	79.29
F7	Free-flowing and white	167.62±9.21	87.00	43.85	87.71
F8	Free-flowing and white	200.20±8.94	90.45	47.71	95.45
F9	Free-flowing and white	216.45±23.20	83.50	42.45	84.91
F10	Free-flowing and white	225.87±26.15	82.00	40.35	80.70

CT: Computed tomography, Mean: Average of 3 trials, mg: Milligrams, SD: Standard deviation, µm: Micrometre

Table 4. % CDR of EPO and hydroxypropyl methylcellulose E50 CT microcapsules in 0.1 N HCl

	% CDR (Mean ± SD)						
Time (min)	0	5	10	15	20	25	30
F1	0	46.52±1.24	76.85±2.15	100.0±0.03	-	-	-
F2	0	44.62±2.67	79.16±1.82	100.0±0.11	-	-	-
F3	0	46.41±1.44	76.27±1.28	100.0±0.14	-	-	-
F4	0	23.42±2.66	52.96±1.85	85.33±2.74	99.21±1.69	-	-
F5	0	14.45±1.21	42.41±2.18	66.30±2.95	84.50±3.46	100.0±0.05	-
F6	0	42.91±1.32	72.33±1.55	100.0±0.40	-	-	-
F7	0	40.86±2.59	70.63±1.06	100.0±0.93	-	-	-
F8	0	38.96±1.84	74.17±1.58	100.0±0.51	-	-	-
F9	0	22.65±2.32	51.88±1.47	79.36±2.88	100.0±0.11	-	-
F10	0	16.29±0.96	38.77±2.77	57.62±2.62	79.82±2.65	98.87±2.21	-

% CDR: Percentage cumulative drug release, EPO: Eudragit® EPO, Mean: Average of 3 trials, min: Minutes, SD: Standard deviation,

integrity, shape, and diameter during the dissolution Figure 8 (A), (B).

Stability studies of CT microcapsules

The optimised CT Eudragit EPO and HPMC E50 microcapsules were subjected to stability studies as per ICH guidelines, and analysed for their physical form, drug content, encapsulation efficiency, and percent CDR. There was no change in the parameters tested, proving their stability.

Evaluation of formulated ODMTs

Evaluation of pre-compression parameters ODMT blend

All the ODMTs formulation blends were free-flowing due to the presence of co-processed ready-to-use tableting excipients, Ludiflash and Pearlitol SD. There was no significant difference between bulk density and tapped density, suggesting that microcapsules had a uniform particle size and similar shapes. Carr's index of less than 20 indicated good compressibility. Hausner's ratio was found to be around 1.18 (<1.25 is good), demonstrating excellent flow property and appropriateness for compression. Table 5 shows the micromeritic properties of the ODMT blend regarding bulk density, tapped density, angle of repose, Carr's Index, and Hausner's ratio.

Evaluation of post-compression parameters of ODMTs

Physical appearance of ODMTs

Upon visual inspection, the formulated ODMTs were found to be tiny, white, biconvex-shaped mini tablets with a smooth texture.

Crushing strength of ODMTs

ODMTs with ludiflash showed crushing strength >4.1 N, and those with pearlitol showed >4.4 N, indicating good strength for handling.

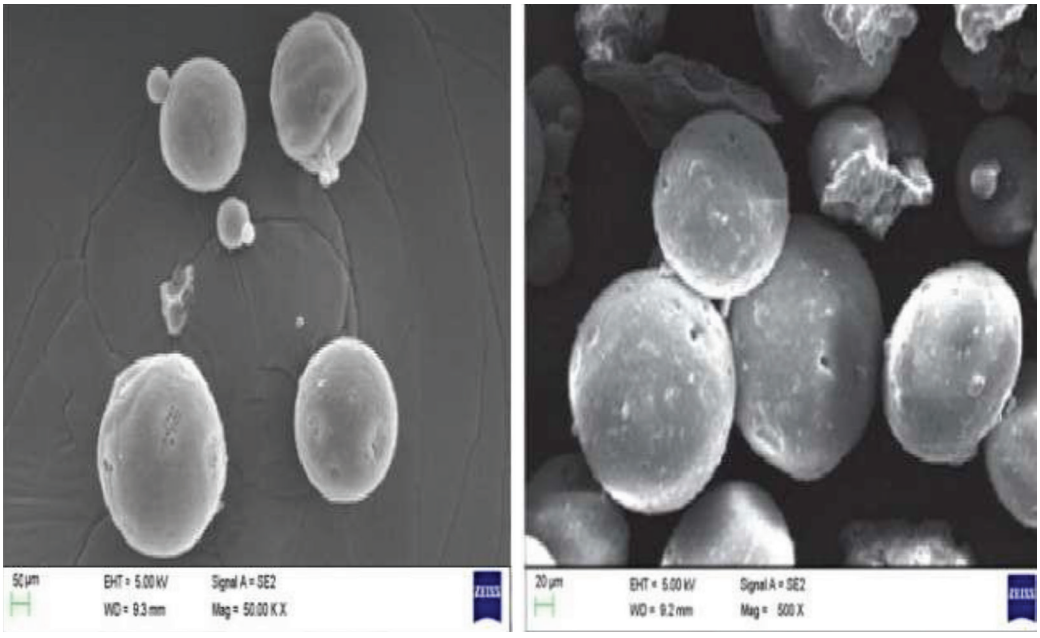

Figure 6. SEM images of optimised CT EPO microcapsules (F3)
CT: Computed tomography, EPO: Eudragit® EPO, SEM: Scanning electron microscopy

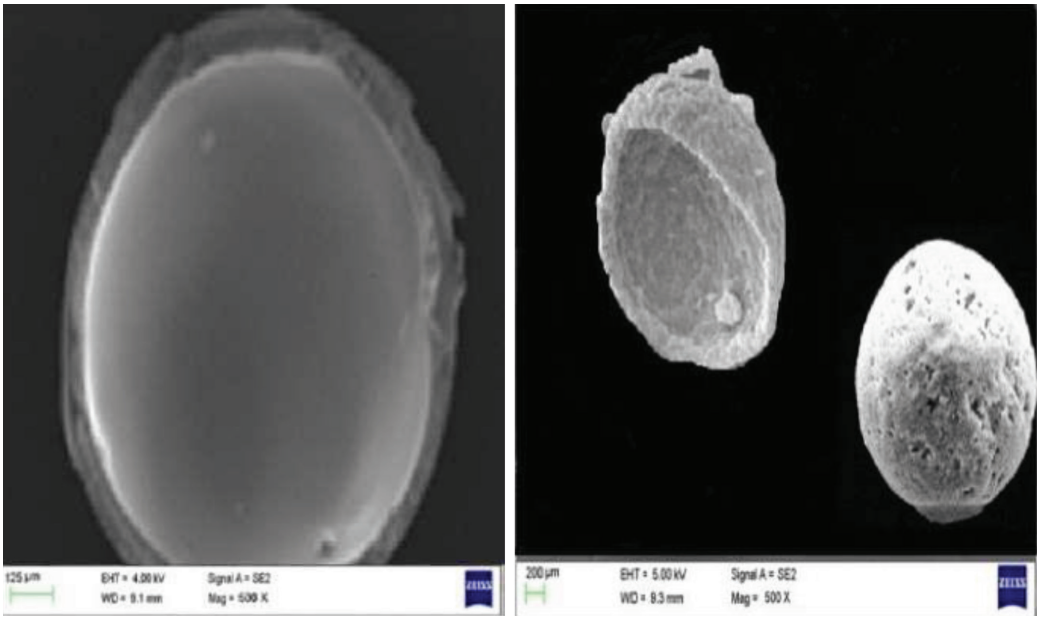

Figure 7. SEM images of optimised CT hydroxypropyl methylcellulose E50 microcapsules (F8)
CT: Computed tomography, SEM: Scanning electron microscopy

F of ODMTs

All the formulated ODMTs showed low friability of <1%, indicating good mechanical strength during handling, packing, and transportation.

Uniformity of mass (weight variation) of ODMTs

Each ODMT was found to weigh around 12 mg±0.44, thereby passing the uniformity of weight variation test.

SWT time of ODMTs

The SWT time of all the ODMTs was found to be less than 10 seconds in phosphate buffer pH 6.8 (simulated salivary fluid), indicating immediate disintegration in the mouth. This achieves the requirement of fast oral disintegration due to the ready-to-use tabletting excipients Ludiflash and Pearlitol 200SD, which disintegrated at pH 6.8.

In vitro dispersion of ODMTs

The *in vitro* dispersion time of all the ODMTs was less than <20 seconds, indicating fast disintegration. This rapid disintegration is attributed to the presence of ready-to-use tabletting excipients Ludiflash and Pearlitol 200SD, which act as super disintegrants.

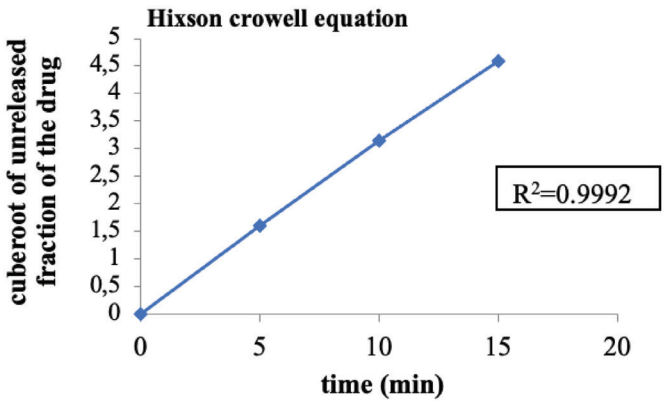

Figure 8 (A). Model fitting curve for optimised CT EPO microcapsules formulation (F3) in 0.1 N HCl

CT: Computed tomography, EPO: Eudragit® EPO, R^2: Regression coefficient

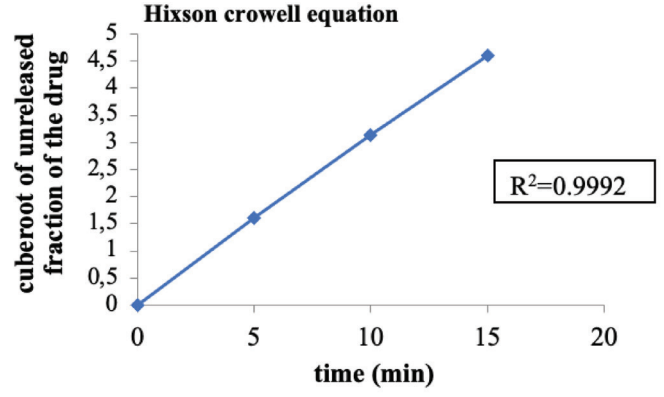

Figure 8 (B). Model fitting curves for optimised CT hydroxypropyl methylcellulose E50 microcapsules formulation (F8) in 0.1 N HCl

CT: Computed tomography, R^2: Regression coefficient

Drug content of ODMTs

The drug content in ODMTs was found to be 1.5 mg/tablet with to drug-to-polymer ratio of 1:1, 2 mg/tablet with to drug-to-polymer ratio of 2:1, and 3 mg/tablet with to drug-to-polymer ratio of 3:1. Thus, the optimised formulation would be FF5 and FF11 with 3 mg drug/tablet. Uniformity of drug content was demonstrated with optimised formulations.

In vitro disintegration test of ODMTs

In vitro disintegration of all the ODMTs was found to be <20 seconds, indicating an immediate disintegration due to the presence of super disintegrants, namely Ludiflash and Pearlitol 200SD.

In vitro dissolution studies of ODMTs

In vitro dissolution studies of optimized ODMTs exhibited an immediate release mechanism (disintegrated <10 secs), characterized by a burst effect in phosphate buffer pH 6.8 (pH of salivary fluid), releasing only drug-entrapped microcapsules, with no drug released in the oral cavity. These microcapsules released the complete drug in a gastric pH of 0.1 N HCl (pH of stomach) within 15 minutes. Thereby concluding that ludiflash and pearlitol dissolve completely in the oral cavity, giving a sweet, smooth, and pleasant mouth feel, which are more acceptable for paediatric patients. It is hence proved that these co-processed ready-to-use tabletting excipients, ludiflash and pearlitol 200SD, were suitable for ODMT formulations aimed at paediatric use. The results were depicted in Table 6A-D.

Drug release kinetics from ODMTs

The R^2 values of the Hixson-Crowell cube root equation for Eudragit EPO ODMTs and those of the Korsmeyer-Peppas equation for HPMC E50 ODMTs were similar to those of their respective microcapsules.

Table 5. Pre-compression parameters of ODMTs blend

Formulation code	Bulk density (g/mL) (Mean ± SD)	Tapped density (g/mL) (Mean ± SD)	Carr's Index (%) (Mean ± SD)	Hausner's ratio (Mean ± SD)	Angle of repose (Mean ± SD)
FF1	0.45±0.018	0.52±0.020	13.46±2.48	1.15±0.036	34.070±0.815
FF2	0.48±0.025	0.55±0.021	12.06±2.23	1.14±0.056	34.430±0.960
FF3	0.49±0.032	0.58±0.063	15.51±1.325	1.18±0.046	33.410±1.600
FF4	0.50±0.028	0.59±0.055	15.25±2.012	1.18±0.061	33.240±1.266
FF5	0.48±0.015	0.55±0.021	12.72±0.837	1.14±0.011	28.080±0.869
FF6	0.51±0.013	0.57±0.024	10.52±0.937	1.11±0.021	26.390±0.415
FF7	0.50±0.018	0.58±0.016	13.79±0.885	1.16±0.036	29.150±0.360
FF8	0.52±0.019	0.59±0.048	11.86±0.822	1.13±0.045	27.250±0.944
FF9	0.49±0.021	0.59±0.087	16.94±2.237	1.20±0.047	31.150±1.392
FF10	0.52±0.026	0.61±0.044	21.15±3.937	1.17±0.051	30.470±1.216
FF11	0.51±0.018	0.60±0.057	15.00±4.012	1.17±0.059	31.150±0.944
FF12	0.50±0.018	0.58±0.064	13.79±4.034	1.16±0.062	33.650±0.562

SD: Standard deviation, Mean: Average of 3 trials, ODMTs: Orally disintegrating mini-tablets

Table 6A. Physical characteristics of our ODMTs

Parameters	Found
Description	White coloured biconvex mini tablets with a smooth texture
Weight of 20 mini tablets	240.00±0.2 mg
Average mass (n=20)	12.00±0.01 mg
Uniformity of the mass (n=20)	12.00±0.01 mg
Diameter (n=20)	2.0±0.00 mm

ODMTs: Orally disintegrating mini-tablets, n: Number of samples tested

Table 6B. Compression force, crushing strength, wetting time and friability of ODMTs

Formulation code	Compression force (kN)	Crushing strength (N) (Mean ± SD)	Wetting time (sec) (Mean ± SD)	Friability (*w/w* %)
FF1	8	4.2±0.8	6±0.4	0.05
FF2	10	4.4±1.7	7±1.7	0.06
FF3	8	4.1±0.9	6±0.6	0.04
FF4	10	4.8±1.2	7±1.4	0.07
FF5	8	4.4±0.6	7±0.5	0.05
FF6	10	4.3±1.6	8±1.2	0.04
FF7	8	4.5±0.8	8±0.3	0.05
FF8	10	4.4±1.4	8±1.4	0.05
FF9	8	4.6±0.9	9±0.5	0.07
FF10	10	4.7±1.8	10±1.4	0.05
FF11	8	4.8±0.8	9±0.7	0.06
FF12	10	4.5±1.5	10±1.9	0.06

kN: KiloNewton, Mean: Average of 3 trials, N: Newton, ODMTs: Orally disintegrating mini-tablets, SD: Standard deviation, Sec: Second

Table 6C. Thickness, uniformity of mass, *in vitro* dispersion time, drug content, *in vitro* disintegration of ODMTs

Formulation code	Thickness (mm) (Mean ± SD) (n=10)	Uniformity of mass (mg) (Mean ± SD) (n=10)	*In vitro* dispersion time (sec) (Mean ± SD) (n=10)	Drug content (mg per tablet) (Mean ± SD) (n=3)	*In vitro* disintegration time (sec) (Mean ± SD) (n=10)
FF1	3.34±0.2	12.1±0.41	13 ±0.7	1.51±0.05	17±0.2
FF2	3.32±0.1	12.1±0.39	16±1.4	1.49±0.04	19±1.6
FF3	3.33±0.1	12.0±0.44	14±0.8	2.0±0.01	18±0.7
FF4	3.31±0.1	12.0±0.40	17±1.9	2.02±0.00	20±1.3
FF5	3.32±0.2	12.0±0.28	14±0.6	3.03±0.04	19±0.4
FF6	3.32±0.2	12.0±0.26	18±1.3	3.01±0.04	21±1.5
FF7	3.34±0.1	12.0±0.29	15±0.4	1.50±0.04	19±0.2
FF8	3.34±0.1	12.0±0.28	18±1.5	1.50±0.05	20±1.3
FF9	3.33±0.2	12.1±0.33	20±0.9	2.0±0.00	20±0.7
FF10	3.34±0.1	11.9±0.32	21±1.2	2.01±0.02	21±1.8
FF11	3.34±0.1	12.1±0.39	19±0.8	3.02±0.05	19±0.9
FF12	3.31±0.2	12.0±0.28	20±1.7	3.01±0.05	20±1.9

Mean: Average of 3 trials, mg: Milligram, mm: Millimetre, n: Number of samples, ODMTs: Orally disintegrating mini-tablets, SD: Standard deviation, Sec: Seconds

Table 6D. *In vitro* dissolution studies of ODMTs in 0.1 N HCl

ODMTs formulations	% CDR in time (minutes) ± SD			
	0	5	10	15
FF1	0	42.21±2.47	70.96±1.28	100.00±0.39
FF2	0	44.97±1.83	71.15±2.98	100.00±0.67
FF3	0	43.76±2.84	69.55±1.21	100.00±0.27
FF4	0	42.34±1.38	68.89±1.53	100.00±0.48
FF5	0	40.57±2.69	67.32±2.54	98.12±1.48
FF6	0	39.94±2.24	66.94±3.76	97.83±2.91
FF7	0	32.76±3.57	65.86±2.64	100.00±0.71
FF8	0	31.96±2.87	64.72±2.74	100.00±0.64
FF9	0	31.12±1.19	65.97±1.56	100.00±0.13
FF10	0	30.65±1.97	64.31±1.64	100.00±0.89
FF11	0	32.11±2.46	64.97±3.39	98.61±1.24
FF12	0	31.85±3.79	63.64±2.81	98.43±0.54

% CDR: Percentage cumulative drug release, SD: Standard deviation, ODMTs: Orally disintegrating mini-tablets

SEM images of ODMTs

Based on the above evaluation parameters, selected ODMTs FF5, FF6, FF11, and FF12 were subjected to SEM studies. Upon visualisation, microcapsules were found to be intact in the ODMTs even after compression. Thus, it is demonstrated that the polymers Eudragit EPO and HPMC E50 can endure the compression pressure owing to their elastic nature. Other excipients also contributed to protecting the microcapsules from being cracked or chipped Figure 9 (A), (B).

Stability studies of ODMTs

The stability studies of ODMTs showed no modification in the appearance, drug content, and *in vitro* dissolution (% CDR). This demonstrates that they were stable when exposed to different atmospheric conditions as per International Council for Harmonisation (ICH) of Technical Requirements for Pharmaceuticals for Human Use guidelines.

DISCUSSION

CT is a broad-spectrum third-generation cephalosporin used to treat a wide range of infections caused by gram-positive and gram-negative organisms. The recommended dose of CT in adults is 400 mg daily, especially in cases of UTI, LRTI, and URTI. The recommended child dose is 8 mg/kg/day. This may be administered as a single daily dose or may be given in 2 divided doses as 4 mg/kg every 12 hours. For infants and toddlers, the recommended dose is 1.5-3 mg/kg given orally twice a day. Presently in the market, only suspensions of CT are available for paediatric use, in strengths of 100 mg/5 mL or 200 mg/5 mL.[23] Thus, administration of these suspensions for infants and toddlers based on their body weight is difficult, leading to inaccurate dosing and posing stability problems.

Hence, there is a need for flexible, dose-adjustable, safe, and stable solid oral dosage forms that could replace the existing liquid dosage forms. There comes the need for ODMTs. These ODMTs combine the concept of a mini tablet and fast-dissolving approaches to formulate a novel child-appropriate dosage form. ODMTs are less than 3 mm in diameter, tiny, very easy to administer, and disintegrate in the mouth as soon as they come in contact with saliva, without the need for water. As they disintegrate in the mouth, they should give a pleasant mouthfeel, which necessitates taste masking of the drug.[24]

Taste masking technology should be compatible with ODMT formulation. This means that the coating should not be broken during compression or dissolved during granulation. Thus, taste masking of bitter-tasting drugs is crucial to the success of ODMT formulation. In an ideal ODMT, the drug property should not affect the tablet property. Based on previous research works and literature survey, the microencapsulation technique was selected for drug taste masking. Microencapsulation is a widely used technique in the pharmaceutical industry to mask the bitter taste of drugs as well as to achieve better bioavailability. During microencapsulation, a thin coating is applied onto small particles of solids, droplets of liquid, or dispersions. In our study, the spray drying technique was employed as a microencapsulation, wherein the bitter drugs are first encapsulated to give free-flowing microcapsules, which are then blended with co-processed ready-to-use tableting excipients and then compressed into minitablets.[25-28]

In our present research, commercially available polymers, namely HPMC E50 and Eudragit EPO, were selected for the microencapsulation technique to mask the bitter taste of the drug. The selected polymers were tasteless and insoluble in the salivary pH and dissolved only at an acidic pH of <5. As a result, the drug was not released in the oral cavity, whereas it was released only after reaching the stomach. Thus, these polymers were employed in our research to control the release of the drug in the stomach and to prevent its release

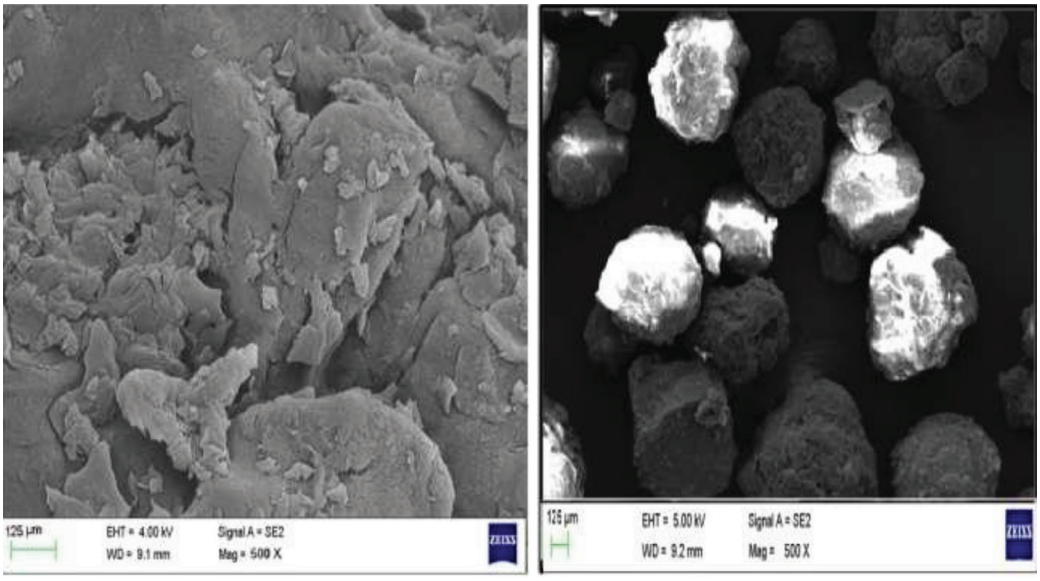

Figure 9 (A). SEM images of ODMTs FF5 and FF6
ODMTs: Orally disintegrating mini-tablets, SEM: Scanning electron microscopy

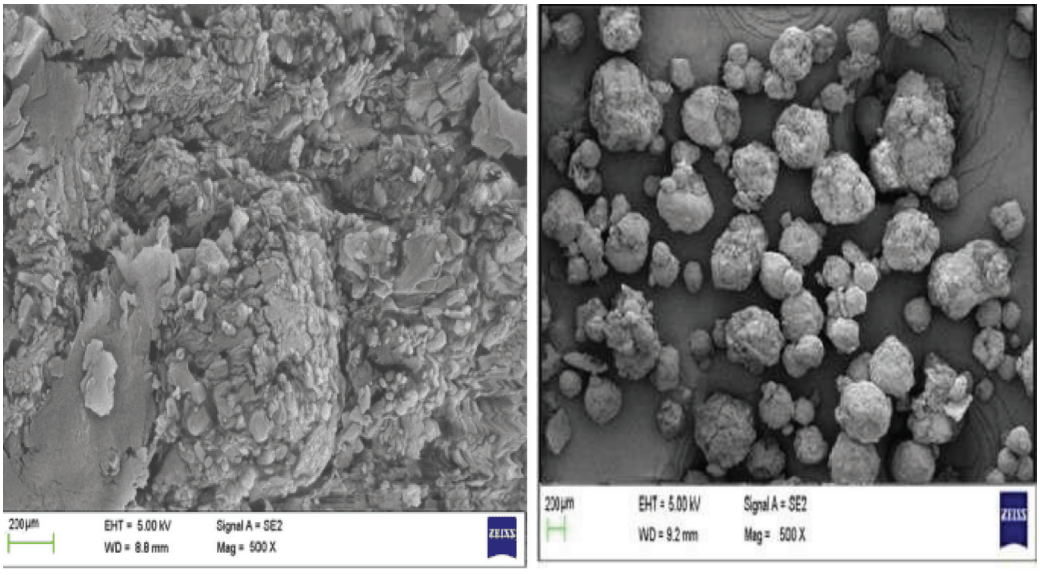

Figure 9 (B). SEM images of ODMTs FF11 and FF12
ODMTs: Orally disintegrating mini-tablets, SEM: Scanning electron microscopy

in the oral cavity.[29,30] After the formulation and optimization of microcapsules, the optimized batch was blended with ready-to-use tableting excipients, namely ludiflash and pearlitol.

Ludiflash is a commercially available, ready-to-use tablet excipient mainly composed of the following:

90% D-Mannitol – Fast-dissolving filler with a mildly sweet taste.

5% Kollidon® CL - SF (Cross povidone) – Superior tablet disintegrant and offers a smooth and creamy mouth feel.

5% Kollidon® SR 30 D (polyvinyl acetate) – Hydrophobic binder for enhanced disintegration.[31]

Pearlitol is also a commercially available, ready-to-use tablet excipient mainly composed of 100% D-mannitol. Both ludiflash and pearlitol have uniform particle size distribution, high bulk density, and provide good flowability. Our research proved that they produce excellent content uniformly, even at high tableting speeds. All the above polymers have been proven safe by European regulatory requirements on products for paediatric use, and hence they are employed in our current research.[32]

Further, our research demonstrated the instant disintegration of ODMTs in the oral cavity due to the presence of ready-to-use co-processed tableting excipients, namely, ludiflash and pearlitol, which resulted in the release of microcapsules rather

than the drug. After swallowing these microcapsules, and only after reaching the gastric region, they release the complete drug within 15 minutes (due to the presence of HPMC E50 and Eudragit EPO), thus achieving taste masking.

Typically, our study demonstrated that, in the first step, disintegration of ODMTs in the oral cavity takes place within a few seconds, releasing taste-masked microcapsules, followed by their swallowing. In the second step, these microcapsules, after reaching the gastric environment, released the complete drug within 15 minutes for drug action. The formulation successfully achieved taste masking (of the bitter drug CT) and also developed novel ODMTs for ease of administration and fast disintegration in the oral cavity for pediatric use.

Present research work revealed that the EPO microcapsules (F3) and HPMC E50 microcapsules (F8) had a drug CT to polymer concentration ratio of 1:3 and were found to be optimized formulations, showing 94.8% and 95.45% drug entrapment efficiency and particle size of 125.45±6.15 µm, and 200.20±8.94 µm, respectively. *In vitro* dissolution of optimized CT EPO microcapsules and CT HPMC E50 microcapsules (F3, F8) exhibited release only at pH in 0.1 N HCl, and followed the Hixson-Crowell cube root equation and the Korsmeyer-Peppas model equation for drug release kinetics. Stability studies proved that the optimized CT EPO and HPMC E50 microcapsules were stable under various atmospheric conditions. Further, ODMTs of CT FF5, FF6, FF11, and FF12 were found to be optimized formulations, containing 3 mg/tablet. Co-processed excipients Ludiflash and Pearlitol 200SD proved to be excellent ready-to-use tableting excipients and acted as super disintegrants. Thus, our ODMTs exhibited fast disintegration (SWT<10 seconds) only at pH 6.8 (phosphate buffer mimicking salivary fluid). The *in vitro* dissolution of ODMTs exhibited release only at phosphate buffer pH 6.8, and followed the Hixson-Crowell cube root equation and the Korsmeyer-Peppas model equation for drug release kinetics. Finally, stability studies of optimized ODMTs proved that the formulations were stable under different atmospheric conditions, as checked per ICH guidelines.

With this technology, a novel solid oral dosage form was developed, satisfying all existing demands for a child-appropriate dosage form: ease of weight-based dose administration without breaking or modifying the tablet, thereby providing flexible dose adaptation. This replaces the less stable liquid oral dosage forms for paediatric groups.

For further research, the suitability of other essential paediatric drugs, as well as potent drugs, should be evaluated.

CONCLUSION

Thus, our ODMTs proved to be an ideal paediatric dosage form with the use of minimal excipients. We were able to formulate a taste-masked, fast-disintegrating, easy-to-swallow, and stable formulation with flexible dose increments. This facilitates the development of a novel oral solid dosage with regard to industrial applicability, with ease of production and bulk scale-up, low transport and storage costs.

Ethics

Ethics Committee Approval: Not required.

Informed Consent: Not required.

Acknowledgments

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Footnotes

Authorship Contributions

Surgical and Medical Practices: V.S., V.G.J., Concept: V.S., V.G.J., Design: V.S., S.B.C., Data Collection or Processing: V.G.J., S.B.C., Analysis or Interpretation: V.S., V.G.J., S.B.C., Literature Search: V.S., S.B.C., Writing: V.S., S.B.C.

Conflict of Interest: The authors declare no conflicts of interest.

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ORIGINAL ARTICLE

Exploring Student Pharmacists' Time Management and Career Planning Attitudes Through a Management Course: An Exploratory Sequential Mixed Methods Study

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ABSTRACT

Objectives: This study aimed to incorporate a pharmacy management course into pharmacy education and explore students' time management and career planning attitudes in relation to this course.

Materials and Methods: This research, conducted between October 2, 2023, and January 12, 2024, employed a mixed-methods design, integrating both qualitative and quantitative research methodologies within a single study framework. Quantitative data were collected using the Sociodemographic Form, Career Futures Inventory (CFI), and Time Management Questionnaire (TMQ). A semi-structured interview form was employed to gather qualitative data. This study included 60 fifth-year pharmacy students. The experimental group (n=30) comprised volunteers enrolled in the "Management in Pharmacy" elective course, while the control group (n=30) consisted of randomly selected volunteers not enrolled in the course.

Results: In the intervention group, pre-test TMQ scores ranged from 56 to 117, yielding a mean score of 80.50±16.12. Post-test scores ranged from 55 to 112, with an average of 86.83±14.09. There was a significant difference in the change in the TMQ scores and Time Attitude scores between the control and intervention groups (p=0.003 and p=0.001, respectively). For the intervention group, pre-test CFI scores ranged from 63 to 116, yielding a mean score of 85.93±15.34. Post-test scores ranged from 68 to 111, with an average of 89.40±12.56. No significant difference was observed in the change in the CFI scores between the control and intervention groups (p=0.311). Student feedback provided insight into the necessity or usefulness, impact, and future suggestions concerning the delivery of this course.

Conclusion: The implementation of educational resources and methodologies aimed at fostering time management abilities and encouraging career planning attitudes from the initial phases of pharmacy education may result in greater outcomes.

Keywords: Career planning attitude, pharmacy students, time management, Türkiye

INTRODUCTION

Pharmacy management skills typically focus on human, technical, and conceptual competencies.[1] One such competency is time management. Pharmacy students must possess effective time management skills to avoid issues when they start their profession.[2-4] Time management skills are crucial for achieving academic success.[5] The level of learning required for success in university is often more demanding and time-intensive than the academic tasks students encountered during high school.[6,7] Time management competencies include tasks such as creating schedules in advance, ranking tasks by priority, studying for exams, and adhering to timelines. Implementing effective study strategies to manage time efficiently can contribute to enhanced academic outcomes. The field of time management

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becomes important for students as the ability to balance leisure time and study hours becomes crucial in preparing for exams.[8] Research indicates that effective time management in education offers numerous benefits, serving as the foundation for many student advisory services. Conversely, poor time management is frequently cited as a major contributor to stress and suboptimal academic performance, often resulting in issues such as inadequate time allocation for tasks, last-minute exam preparation, and missed deadlines imposed by academic staff.[9,10]

Time management also enables students to effectively use their time and resources to achieve career goals. Career planning, an essential component of time management and professional development, constitutes an ongoing process of self-evaluation and goal-setting that supports students in achieving their career aspirations.[11] Planning is essential for effective time management in the context of career management. This process entails identifying the most critical tasks and allocating time accordingly, enabling individuals to concentrate on high-impact activities that directly support their career objectives while reducing time spent on less essential or time-consuming tasks.[12] Career management encompasses an individual planning, developing, and realizing their career path throughout their working life. This process involves making strategic decisions, taking steps to achieve professional goals, and maximizing an individual's potential. It also includes identifying the individual's talents, interests, and goals, followed by creating a plan to accomplish them. Since goals can change over time and conditions may evolve, career management requires continuous development.[13] Career management is a dynamic and multifaceted process that involves maintaining professional development and shaping one's career paths. This process encompasses a series of intentional actions, from self-assessment to setting career goals, improving skills, building networks, and making career decisions. Effective career management assists in making conscious choices, adapting to changing conditions, and sustaining long-term career goals.[14] Active participation in this process by students can enhance employability, job satisfaction, and overall well-being.[15] It equips individuals with the necessary tools and strategies to thrive in their chosen careers and adapt to the evolving demands of the business world. Therefore, providing career management education to university students is essential.[16,17]

With increasing competition in the field of pharmacy, extracurricular experiences have become increasingly important for students. Pharmacy faculties are working to enhance their curricula to aid students in cultivating these skills and guiding their professional trajectories.[18] This process requires students to recognize their areas of interest and integrate these interests into their post-graduation goals. Nonetheless, students might struggle to concentrate on these issues until they participate in advanced pharmacy practice. At this point, providing additional opportunities and experiences for professional development can help students build a solid foundation for their post-graduation career goals.[19,20]

There is a limited number of management-related courses in pharmacy faculty education programs, and management issues are not given sufficient importance in undergraduate education in Türkiye. Karahan et al.'s[21] study revealed that 33.65% of pharmacists reported having taken courses related to management. However, only 8.33% of those who had received management training indicated that they had taken such courses during their undergraduate education. Moreover, to the best of our knowledge, there has been no previous research that specifically investigates the impact of a pharmacy management course on time management and career planning. Therefore, the aim was to incorporate a pharmacy management course into pharmacy education and explore students' time management and career planning attitudes in relation to this course.

MATERIALS AND METHODS

Study setting

This study was conducted at the Faculty of Pharmacy, Atatürk University. This faculty, located in eastern Türkiye with deep historical roots, offers a five-year undergraduate degree in pharmacy. At the time of this study, only one elective course in the pharmacy curriculum specifically addressed the development of management skills.

Study design

In this research, a mixed-method approach was utilized, characterized by the integration of qualitative and quantitative research methodologies.[22] Specifically, an exploratory sequential mixed-method[23] design was utilized. This methodological coherence allows for a broader interpretation of the results and a more nuanced evaluation of the impact of the management course on enhancing time management and career planning attitudes among pharmacy students.[24,25] The quantitative data collection comprised the use of surveys and measurement instruments to evaluate students' attitudes regarding time management and career planning, thus providing a wide-ranging perspective. Conversely, the qualitative data collection was carried out through semi-structured interviews, which offered in-depth insights, enabling a deeper comprehension of students' experiences, perceptions, and emotional reactions.

Participants

This study involved fifth-year pharmacy students enrolled in the Management in Pharmacy course during the fall semester of 2023-2024 at the Faculty of Pharmacy, a public university in Türkiye that provides a five-year undergraduate pharmacy education. This study was conducted between October 2, 2023, and January 12, 2024. The "Management in Pharmacy" course had 43 fifth-year pharmacy students enrolled. Among them, 30 students voluntarily participated in the study, forming the experimental group for management skills training. Subsequently, additional fifth-year pharmacy students who did not enroll in the course were invited to participate in the study as part of the control group. Among those who voluntarily agreed to participate, 30 students were randomly selected

using a simple lottery method to constitute the control group. Therefore, 60 participants were included in this study. The participant selection process is illustrated in Figure 1.

Study tools

Quantitative data collection comprised the Sociodemographic Form, Career Futures Inventory (CFI), and Time Management Questionnaire (TMQ). To gather qualitative data, a semi-structured interview format was used.

Sociodemographic form

The researchers developed the sociodemographic form used in this study. It included information on demographic characteristics, such as the students' age and gender.

CFI

The CFI, developed by Rottinghaus et al.,[27] is designed to assess positive career planning attitudes. Kalafat[28] established the validity and reliability of this scale in the Turkish context. The CFI consists of 25 items organized into three subdimensions: Career Adaptability (CA), Career Optimism (CO), and Perceived Knowledge of the Job Market (PK). Respondents rate each item using a five-point Likert-type scale. In the present study, the Cronbach's alpha values were found to be 0.82, 0.85, and 0.76 for the CA, CO, and PK subdimensions, respectively, and 0.90 for the overall scale. Higher scores on the CFI indicate more positive attitudes toward career planning.

TMQ

The TMQ, created by Britton and Tesser,[29] is utilized to evaluate time management practices among university students. The scale's validity and reliability for the Turkish population were established by Alay and Koçak.[30] The TMQ consists of 27 items categorized into three subdimensions: Time Planning (TP), Time Attitudes (TA), and Time Wasters (TW). Respondents are asked to rate their answers using a scale from Never (1) to Always (5). Higher scores on both the overall scale and its subdimensions signify more effective time management practices. In this study, the Cronbach's alpha values were 0.89, 0.72, and 0.71 for the TP, TA, and TW subdimensions, respectively, and the overall scale reliability was also 0.89.

Semi-structured interview form

A semi-structured interview guide (Appendix 1) was developed to collect information regarding the participants' experiences and suggestions concerning the impact of the pharmacy management course. This guide was formulated based on the study's objectives and the collective expertise of the research team.

Intervention procedure and data collection

At the start of the semester, students were briefed on the study's purpose, emphasizing the voluntary nature of their participation and clarifying that personal data, such as school ID and names,

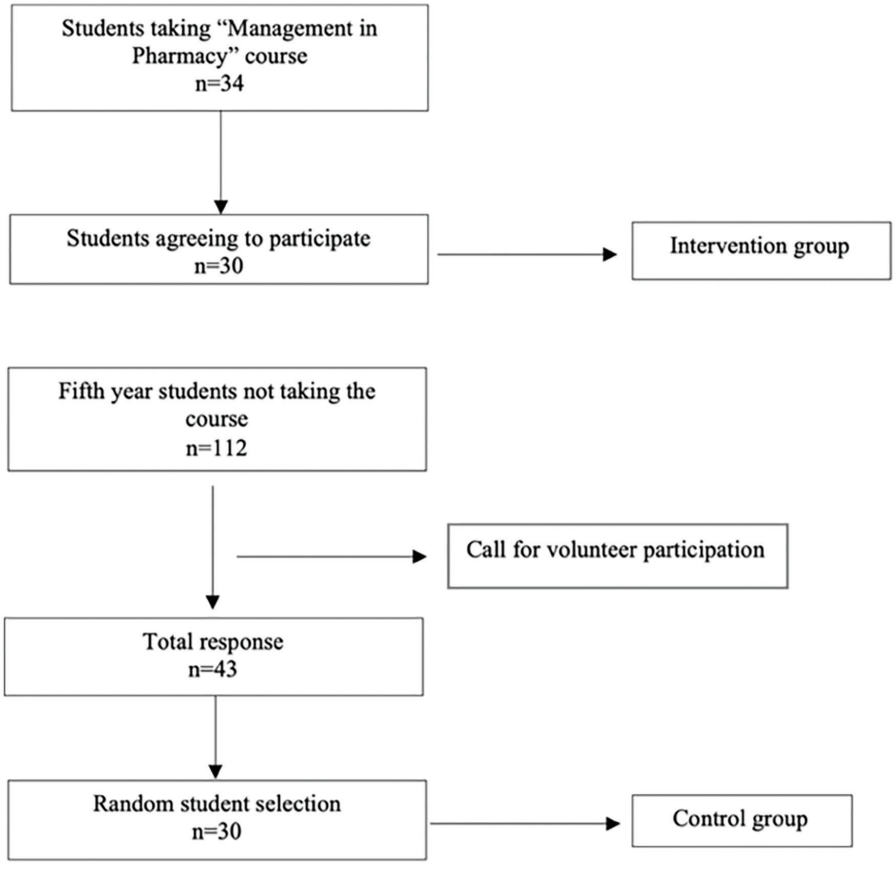

Figure 1. Flowchart of the participant recruitment process

was unnecessary. Each student was asked to write a nickname. All participants wrote their pseudonyms both in the pre-test and post-test, so that comparisons could be made. Importantly, they were assured that their decision to participate or not would not impact their course passing grades. Pre-test evaluations were administered before the commencement of the session, while post-test evaluations took place after the final week. Before the initiation of the intervention procedure, the scales (CFI and TMQ) designated for the research were administered as pre-tests to determine the baseline status of the students in both the experimental and control groups. The content of the Management in Pharmacy course was developed concerning a study conducted by Çingöl and Karakaş,[31] as it includes management skills training that can also be used in the field of pharmacy. It encompasses topics related to the management and leadership processes, functions, and tools. This course was integrated as an elective course into the fifth year of the pharmacy curriculum at the university where the study was conducted. The course was delivered by a single educator with expertise in social skills, ensuring that the content was effectively tailored to promote essential competencies such as time management and career planning among pharmacy students. Over the study period, the course was delivered in a classroom setting for two hours per week for 14 weeks. Appendix 2 provides an overview of the educational content covered in the course. After the conclusion of the semester, the intervention group undertook post-tests on the relevant scales. The control group, which did not receive any training, also engaged in the post-tests for the same scales. Data collection instruments were administered to students during the pre-test, mid-test, and post-test phases, requiring approximately 25 minutes for completion. Qualitative methods were employed following the collection of quantitative data, aligning with the constructivist-interpretive paradigm. Collecting the qualitative data involved in-depth interviews. The study team developed and approved the interview guide. The interviews were conducted after the completion of the course but prior to the assignment of grades. This timing allowed participants to fully experience the course while minimizing any potential bias related to academic evaluation. The interview questions aimed to understand how the students perceived the management course (Appendix 1). All interviews were conducted in person by the first author, lasting between 20 and 25 minutes each. With participants' permission, the sessions were recorded digitally and transcribed word-for-word. In addition, non-identifiable field notes were made to support the reflexivity of the experiences. To ensure confidentiality, each participant will be designated by the letter "S" followed by their unique identification number (S1-30).

Statistical analysis

Quantitative data

Statistical analyses were carried out using IBM SPSS Statistics 26 software. The results were analyzed using frequency tables and descriptive statistics. The normality of the data was assessed using the Shapiro-Wilk test. Given the normal distribution of the data, parametric methods were employed. The independent samples *t*-test was used for comparisons between two distinct groups, whereas the paired-sample *t*-test was used for comparisons within dependent groups. Additionally, Pearson's correlation coefficient was applied to evaluate the associations between variables.

Qualitative data analysis

Given that one of the study's objectives was to explore the perceptions regarding the enhancement of time management and career planning through the management course. The qualitative aspect of the research was conducted exclusively with students from the intervention group who had completed this course. The discussions that were recorded were transcribed word-for-word and then reviewed by participants to verify their accuracy. Two researchers independently examined the transcripts to develop a comprehensive understanding of the content and to establish initial codes using thematic analysis. Afterward, the researchers collaborated to compare the identified themes, revisited the data to reach a consensus, and confirmed thematic saturation through a strategy of code and recoding. Core themes were evaluated about students' views on the impact of the "Management in Pharmacy" course on their time management and career planning. Maxqda Analytics Pro, a qualitative software package, was used to manage qualitative data.

Ethical consideration

This study was carried out in alignment with the principles set forth in the Declaration of Helsinki. Ethical clearance was obtained from the Ethics Committee of the Atatürk University Faculty of Pharmacy (approval number: B.30.2.ATA.0.01.00/720, dated: 07.09.2023). Written authorization was received from the institution hosting the study, and informed consent was acquired from all participating students.

RESULTS

Demographics

All the students (n=60) in the study were in the pharmacy program, and all participated in the quantitative arm of the study. Among the participants in the control group (n=30), 21 (70%) and 16 (53.3%) of the participants in the experimental group (n=30) were female. The average age of the control group was 23.00 (0.79), while the average age of the intervention group participants was 23.67 (1.37). The experimental group participated in this qualitative study (Table 1).

Table 1. Student demographics

Demographic	Control	Intervention
Age, mean (SD) Range	23.00 (0.79) 22-25	23.67 (1.37) 22-27
Gender		
Female	21	16
Male	9	14

SD: Standard deviation

Quantitative

Comparison of CFI and TMQ pre- and post-test scores between control and intervention groups

In the pre-intervention assessment, the TMQ and CFI scores were compared between the control and intervention groups, revealing no significant differences. The baseline scores were comparable between the control group (84.07±11.95 for TMQ and 84.20±14.99 for CFI) and the intervention group (80.50±16.12 for TMQ and 85.93±15.34 for CFI). Furthermore, statistical analysis showed no significant differences between the control and intervention groups for both the TMQ and CFI scores (p=0.063 and p=0.408, respectively). These findings indicate a similar baseline level between the two groups.

In the control group, TMQ scores ranged from 66 to 106 in the pre-test, with a mean score of 84.07±11.95. In the post-test, scores varied between 57 and 111, with an average of 86.23±11.78. In the intervention group, pre-test TMQ scores ranged from 56 to 117, yielding a mean score of 80.50±16.12. Post-test scores ranged from 55 to 112, with an average of 86.83±14.09. There was a significant change in the TMQ and Time Attitude scores between the control and intervention groups (p=0.003 and p=0.001, respectively). In the control group, the CFI scores ranged from 56 to 110 in the pre-test, with a mean score of 84.20±14.99. In the post-test, scores varied between 61 and 115, with an average of 85.73±13.39. For the intervention group, pre-test CFI scores ranged from 63 to 116, yielding a mean score of 85.93±15.34. Post-test scores ranged from 68 to 111, with an average of 89.40±12.56. No significant difference was observed in the change in the CFI scores between the control and intervention groups (p=0.311). Following the completion of the "Management in Pharmacy" course, a significant increase was observed in CA, TP, TA, TW, and TMQ scores among the students in the intervention group (p<0.05) (Table 2).

Qualitative

Three main themes and their subsequent sub-themes emerged from the dataset (Table 3): development of career management skills, factors affecting career management skills, and development of time management skills.

Theme 1. Development of career management skills

Some students mentioned that they gained detailed information about their professions, while others stated that they began to shape their careers.

Subtheme 1.1. Learning the work areas of the profession

Some students mentioned that they learned about various work areas within their future professions, and some highlighted redirecting towards a new work area. The sample expressions are as follows:

"I am confident that the comprehensive career management I have seen within the scope of the course has positioned me a step or two ahead. I can see what exists not only in a single field, but also in other areas of my profession. I believe that being able to see these aspects is crucial for career planning." (S-3)

"From the day I started this programme, I knew that my career would continue in a community pharmacy. After taking this course, I realized that this profession consists of different work areas." (S-9)

Table 2. Comparison of pre- and post-test scores in CFI and TMQ between control and intervention groups

Variable	Control group		Intervention group		
	Pre-test (n=30)	Post-test (n=30)	Pre-test (n=30)	Post-test (n=30)	
	X ± SD	X ± SD	X ± SD	X ± SD	*p*
CA	40.17±8.51	40.80±6.49	40.27±6.45	42.77±6.10	0.185[a] 0.022[b]*
CO	39.67±6.77	39.30±6.09	36.43±9.52	36.83±8.53	0.660[a] 0.754[b]
Perceived knowledge of the job market	9.37±2.16	9.63±2.57	9.23±2.71	9.80±1.88	0.576[a] 0.156[b]
CFI	84.20±14.99	85.73±13.39	85.93±15.34	89.40±12.56	0.311[a] 0.107[b]
TP	53.00±9.31	52.57±8.76	48.10±11.77	51.23±10.96	0.062[a] 0.037[b]*
TA	22.37±3.72	21.83±3.21	20.73±4.47	23.13±3.32	0.001[a]* 0.002[b]*
TW	12.70±2.41	12.83±2.81	11.67±3.30	12.47±3.05	0.202[a] 0.047[b]*
TMQ	84.07±11.95	86.23±11.78	80.50±16.12	86.83±14.09	0.003[a]* 0.002[b]*

[a]An independent-samples *t*-test. [b]Paired sample test for intervention group. *p <0.05 indicates statistically significant differences. CA: Career Adaptability, CO: Career Optimism, CFI: Career Futures Inventory, TP: Time Perspective, TA: Trait Anxiety, TW: Tolerance for Workload, TMQ: Time Management Questionnaire

Table 3. Themes and sub-themes

Themes	Sub-themes	Categories	Description	Representative statement
1. Development of career management skills	1.1. Learning the work areas of the profession		Exploring the diverse work domains within the profession to gain a comprehensive understanding of its scope and opportunities	"I am confident that the comprehensive career management I have seen within the scope of the course has positioned me a step or two ahead. I could see what exists not only in a single field but also in other areas of my profession. I believe being able to see these aspects is crucial for career planning." (S-3)
	1.2. Planning for post-graduation		Developing strategic plans for life after graduation	"In this course, you opened up another field for me: Academia. And I have decided that right after graduation, I will prepare for the relevant exams." (S-10)
2. Factors affecting career management skills	2.1. Impact of time management		The influence and effectiveness of time management on career management skills.	"I have come to understand the connection between career management and time management, and I gained this awareness through this course. While I had separate thoughts on career management and time management before, I did not realize the depth of their connection as detailed in the course. Thanks to this course, I believe there is a significant correlation between career management and time management." (S-12)
	2.2. Impact of environmental factors		The influence of environmental factors on the development of career management skills	"I unfortunately do not see career management that way. Yes, we are talking about this issue, but it is not significant. Because it's not in our hands. If we are rich enough, it's one thing; if we are lucky enough, it's another. Luck resides wherever it smiles. Let's say luck didn't smile at me, or let me put it this way: if luck smiled at me from all sides (which is impossible), why would we even be talking about this? I don't get to choose." (S-14)
3. Development of time management skills	3.1. Understanding the importance of time management		Grasping the significance of effective time management	"Before this course, I didn't give much importance to time management; I used to plan the topics I needed periodically. However, I realized that a significant part of my life was wasted in terms of time." (S-16)
	3.2. Impact on social life	Keeping a planner	Embracing the practice of maintaining a planner	"I have benefited from time management in my social life as well, and I started keeping a planner because of its positive impact." (S-3)
		Enhancing stress management	Improving stress management	"I used to be generally stressed and anxious. I realized that the main reason for this was not making plans in my life and procrastinating on tasks until the last minute. Although I couldn't organize all my tasks after classes, I began doing urgent, important tasks on time. There has been a significant decrease in my stress issues." (S-13)

Table 3. Continued

Themes	Sub-themes	Categories	Description	Representative statement
3. Development of time management skills	3.2. Impact on social life	Utilizing leisure time effectively	Making the most of leisure time by using it effectively	"Before the course, I used to spend my free time by myself after taking care of daily tasks. However, now I have filled that free time more productively." (S-9)
		Coping with procrastination	Developing strategies to overcome procrastination	"Before this course, I had a habit of constantly procrastinating. Regardless of how important something was, I always waited until the last minute. Thanks to this course, I have gained the ability to manage my responsibilities more comfortably by spreading them over time and avoiding last-minute rushes." (S-24)
		The ability to say "no"	Mastering the art of saying "no" for better time management	"For instance, I have learned to say "no" to offers or activities that arise unexpectedly or that I do not want to engage in, especially when they deviate from my plans." (S-1)
	3.3. Enhancing academic success		Optimizing academic success through the effective implementation of time management strategies	"In the 5th grade, many classes involved presentation assignments and group work. With the knowledge gained from this course, I tried to use my time more efficiently by incorporating progress plans. I learned that dividing responsibilities into days and weeks according to the final submission date of assignments could be beneficial." (S-5)

1.2. Planning for post-graduation

Some students mentioned that they had made decisions and plans regarding what they would do after graduation. The sample expressions are as follows:

"In this course, you introduced another field for me: Academia. I have decided that right after graduation, I will prepare for the relevant exams." (S-10)

Theme 2. Factors affecting career management skills

Most students expressed that their career management was influenced by time and environmental factors.

Subtheme 2.1. Impact of time management

Some students suggested that time and career management were interconnected. They pointed out that effective time management had positive contributions to career management. The sample expression is as follows:

"Through this course, I have come to understand the connection between career management and time management." While I had separate thoughts on career management and time management before, I did not realize the depth of their connection, as detailed in the course. Thanks to this course, I believe there is a significant correlation between career management and time management (S-12).

Subtheme 2.2. Impact of environmental factors

Despite their desire to be successful in career management, most students expressed concerns about effectively managing their careers due to external factors such as luck and money. The sample expression is as follows:

"I do not see career management that way, unfortunately. Yes, we are discussing this issue, but it is of limited significance. This is beyond our control. If we are rich enough, it is one circumstance. If we are lucky enough, it is another. Wherever luck smiles, that is, where. Well, let us say luck did not smile at me, or let me put it this way: if luck were to smile at me from all sides (which is impossible), why are we even talking about this? I don't get to choose." (S-14)

Theme 3. Development of time management skills

Almost all students mentioned that after their educational experiences, they realized the importance of time management and talked about its positive impacts on both their social and academic lives.

Subtheme 3.1. Understanding the importance of time management

Most of the students realized they were wasting time and accepted their shortcomings in this regard. The sample expressions are as follows:

"Before this course, I didn't give much importance to time management; I planned the topics periodically." However, I realized that a significant part of my life was wasted." (S-16)

"Before taking this course, I was unaware that I was using my time inefficiently. In the course, I realized how to manage my time and became aware that social media was consuming it." (S-23)

Subtheme 3.2. Impact on social life

Most students mentioned that they had started effectively managing their time. They also discussed its positive contributions to their social lives, such as better stress management, effective use of free time, and coping with procrastination more efficiently.

Most students stated that they are trying to live a more planned life than before and have started keeping a planner, using digital or physical planners. The sample expression is as follows:

"I have benefited from time management in my social life as well, as a result, I started keeping a planner because of its positive impact." (S-3)

Enhancing stress management: Some students claimed that they did not manage their time well, and this led to time-related stress. However, after starting to live more planned lives, they asserted that their stress levels had decreased. The sample expression is as follows:

"I used to be stressed and anxious. I realized that the main reason for this was my lack of planning and procrastination on tasks until the last minute. Although I could not organize all my tasks after class, I started to perform important and urgent tasks on time. There has been a significant decrease in my stress levels." (S-13)

Utilizing leisure time effectively: Some students mentioned that they spent their free time more effectively. The sample expression is as follows:

"Before the course, I used to spend my free time on myself after taking care of daily tasks. However, now I have filled my free time more productively." (S-9)

Coping with procrastination: A few students mentioned that they had started doing things they used to procrastinate on, and no longer left their tasks to the last minute. The sample expression is as follows:

"Before this course, I had the habit of constantly procrastinating. Regardless of how important something was, I waited until the last minute. Thanks to this course, I have gained the ability to manage my responsibilities more comfortably by spreading them over time and avoiding last-minute rushes." (S-24)

The ability to say "No": Some students mentioned that they can now say "No" to accomplish the tasks they planned. The sample expression is as follows:

"For instance, I have learned to say "No" to offers or activities that arise unexpectedly or that I do not want to engage in, especially when they deviate from my plans." (S-1)

Subtheme 3.3. Enhancing academic success

Some students emphasized the positive effects of effective time management on their academic lives. The sample expression is as follows:

"In the 5th grade, many classes involved presentation assignments and group work. With the knowledge gained from this course, I tried to use my time more efficiently by implementing structured progress plans. I learned that dividing responsibilities into days and weeks based on the final submission date of assignments could be beneficial." (S-5)

DISCUSSION

This study represents the first investigation in Türkiye focusing on the effects of the time management and career planning components of the pharmacy management course. The students exhibited significant improvements in their overall scores for TP, time attitude, and TW and overall in the TMQ. This finding indicates that the Management in Pharmacy course played a role in enhancing the time management skills of pharmacy students. Although there was a notable increase in the scores related to CA, no significant changes were observed in CO, perceived knowledge of the job market, or CFI scores. The nature of this study, focusing on 5th-year pharmacy students who were a few months away from graduation and experienced high job anxiety, might have influenced the study's findings. Student feedback provided insight into the need/utility, impact, and future suggestions concerning the delivery of this course.

Rather than teaching job-specific skills, time management training aims to enhance cognitive skills, enabling individuals to assess situations and develop the cognitive processes needed to address various topics or issues. The findings reported in this article suggest that time management training programs can lead to an understanding of fundamental principles and, as evaluated by the majority of participants, can result in improvements in relevant skill areas.

The results of a study conducted with healthcare service students, in which management training was provided, indicate the necessity of incorporating time management education into future stages of the health sciences curriculum.[32] Considering this finding, the current study was conducted with pharmacy students in the final year of their undergraduate education.

In the intervention group of this study, statistically significant differences were found between the pre-test and post-test scores in time management and all its subdimensions. In a similar investigation by Çingöl and Karakaş,[31] involving nursing students, significant variations were noted in the 'TP' subdimension and the overall time management scores between the pre-test and post-test assessments, with post-test scores showing considerable improvement. Conversely, no significant differences were found between pre-test and post-test scores for the 'TA' and 'TW' subdimensions.[31]

In this study, students recognized the significance of effective time management. In a similar qualitative investigation carried out with pharmacy students in 2017, some students perceived time management as a prerequisite for holding a position, whereas others viewed it as a skill to be developed for future use in their roles as pharmacists.[33]

In this study, students have also asserted that time management significantly impacts their academic lives. The literature also indicates that the time pharmacy students allocate to their studies affects learning. A significant relationship has been demonstrated between the overall grade point average and

short-term planning skills, as well as attitudes toward time management.[4] This suggests that teaching time management skills to students may be advantageous.

In the qualitative segment of the research, the students conveyed that their stress and anxiety levels decreased as they managed their time well. In support of this finding, a study by Zhang et al.[34] indicated that healthcare students subjected to a structured time management training program experienced significant enhancements in their time management abilities, along with a decrease in anxiety levels.

The literature supports the idea that college students can derive positive results from intervention programs designed to increase their career awareness.[35-41] In a study carried out with nursing students, significant variations were identified between the pre-test and post-test scores regarding the sub-dimensions of "CA" and "CO," along with the overall scores. The post-test assessments reflected markedly higher values.[31] In this study, within the intervention group, a significant increase was observed in the "CA" sub-dimension between the pre-test and post-test scores. This suggests that the management course enhances students' confidence in dealing with developmental challenges, making them more optimistic about their professional future in the face of tasks and unexpected changes in their professional life.

This study presents several limitations. Firstly, data were collected using self-report scales, which may introduce the of expectation bias. However, steps were taken to mitigate this bias by ensuring the anonymity of respondents. Secondly, this research was restricted to one institution and a specific group of students, which may influence the generalizability of the outcomes. Furthermore, the sample size was relatively small, given that elective courses at the institution typically involve about 35 students. Thus, it is recommended that this study be repeated with a larger sample size to achieve more substantial findings. Finally, there was a 14-week interval between the pre- and post-assessments. This study did not assess the long-term effects on time and career management, warranting further investigation into the sustainability of these skills beyond course duration.

In the contemporary world, characterized by rapid changes, time is a crucial resource. Given that adults dedicate nearly one-third of their time to professional life, the significance of effective time management and career planning is paramount. In this context, pharmacy education has the potential to play a pivotal role in nurturing time management and career adaptation skills among pharmacy students.[30] It can function as a key element in their ability to navigate the diverse changes and progressions that may occur in their personal and professional lives. Given the dynamic nature of the job market and the increasing necessity for guidance and information about career pathways and employment possibilities, it is crucial to enhance the current educational framework.[9] Enriching the curriculum to encompass global changes and contemporary health policies can better equip pharmacy students to meet the challenges and seize the opportunities of an ever-evolving professional landscape.

CONCLUSION

The results of this study suggest that the Management in Pharmacy course improves the time management skills of fifth-year pharmacy students, while significantly enhancing their adaptability regarding career-planning attitudes. Additionally, a positive correlation was observed between time management and career planning, with the Management in Pharmacy course strengthening this relationship. The intervention also highlighted the positive impact on students' learning experiences, fostering a rich environment for discussions and group learning. These results provide insights for further exploration of these pivotal concepts for pharmacists in future research. The incorporation and continuous implementation of educational resources and methodologies aimed at fostering time management abilities and encouraging career planning attitudes from the initial phases of pharmacy education may result in more profound outcomes. Numerous factors may affect both time management and career planning. Consequently, further comprehensive research is necessary to explore these factors and evaluate their influence.

Ethics

Ethics Committee Approval: This study was carried out in alignment with the principles set forth in the Declaration of Helsinki. Ethical clearance was obtained from the Ethics Committee of the Atatürk University Faculty of Pharmacy (approval number: B.30.2.ATA.0.01.00/720, dated: 07.09.2023).

Informed Consent: Written authorization was received from the institution hosting the study, and informed consent was acquired from all participating students.

Footnotes

Authorship Contributions

Concept: E.U.D., Design: E.U.D., Data Collection or Processing: E.U.D., R.E., Analysis or Interpretation: E.U.D., R.E., Literature Search: E.U.D., R.E., Writing: E.U.D., R.E.

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Appendix 1. Interview questions

1. How would you define time management?
2. How would you define career management?
3. Did the course deliver what you were expecting it to?
4. What has been the benefit of this training program?
5. How did the course impact your time management skills?
6. How did the course impact your career management skills?
7. Is there anything else you would like to share or add on these topics?

Appendix 2. Content of the management in pharmacy course

Sessions	Session content	Methods used in the session
Week 1	Management Concepts and Theories	Theoretical lecture, Brainstorming, and Questions and Answers
Week 2	Management Process and Management of Pharmacy Services	Theoretical lecture, Brainstorming, and Questions and Answers
Week 3	Healthcare System, Health Policies, and Legal and Ethical Regulations Regarding Pharmacy	Theoretical lecture, Brainstorming, and Questions and Answers
Week 4	Time Management	Theoretical lecture, Brainstorming, and Questions and Answers Case study discussion and Individual Study (individual time analysis and time matrix creation)
Week 5	Problem-Solving and Decision Making	Theoretical lecture, Brainstorming, and Questions and Answers Case study discussion and Group work (identifying problems related to clinical processes, coming up with options, and deciding the solutions)
Week 6	Career Planning and Development	Theoretical lecture, Brainstorming, and Questions and Answers Case study and discussion, Individual study (self-strengths, weaknesses, opportunities, and threats analysis, goal setting and action plan creation, CV preparation, and researching career options)
Week 7	Communication Management Conflict Management	Theoretical lecture, watching a movie, and Questions and Answers Document review (official forms, meeting report, etc.), case study, and discussion
Week 8	Leadership (Power, Authority, and Influence) -Motivation and Job Satisfaction	Theoretical lecture, watching a movie, and Questions and Answers
Week 9	Human Resources Management	Theoretical lecture, Brainstorming, Questions and Answers, Case study and discussion, Group work (determining workload on nurses and creating a shift schedule), and document review (job definitions and job requirements forms)
Week 10	-Care Delivery Methods in Nursing -Team Work	Theoretical lecture, Brainstorming, Questions and Answers, Case study and discussion, and Group work (identifying problems and coming up with solutions)
Week 11	Change Management	Theoretical lecture, Brainstorming, Questions and Answers, Case study and discussion, Group work (change in the plans)
Week 12	Crisis Management	Theoretical lecture, Brainstorming, Questions and Answers, Case study and discussion, and Document review (hospital emergency action plan)
Week 13	Quality Management	Theoretical lecture, Brainstorming, Questions and Answers, Case study and discussion, Document review (quality documents, error reports)
Week 14	Managerial Ethics	Theoretical lecture, Brainstorming, Questions and Answers, Case study and discussion, and Literature review