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SEMI-SOLID EXTRUSION 3D PRINTING OF FUNCTIONALIZED POLYETHYLENE OXIDE GELS LOADED WITH 1,2,3-TRIAZOLO-1,4-BENZODIAZEPINE NANOFIBERS AND VALINE-MODIFIED MOTHERWORT (*LEONURUS CARDIACA* L.) DRY EXTRACT

Iryna Botsula, Oleh Koshovyi, Igor Kireyev, Maryna Mazur, Valentyn Chebanov, Jyrki Heinämäki, Ain Raal

Anxiety disorders are the most prevalent psychiatric disorders and are associated with a high burden of illness. Combining synthetic and native-origin compounds in treating such disorders could provide true benefits in terms of therapeutic efficacy. In the present study, we combined triazolobenzodiazepine and motherwort (Leonurus cardiaca L.) dry extract for such applications.

The aim. *The aim of this study was to develop aqueous polyethylene oxide (PEO) composite gels loaded with 1,2,3-triazolo-1,4-benzodiazepine nanofibers and a valine-modified motherwort herb dry extract for semi-solid extrusion (SSE) 3D printing. The printability of such gels and the physicochemical properties of the final 3D-printed drug preparations were investigated.*

Materials and methods. *A new drug substance, 1,2,3-triazolo-1,4-benzodiazepine (MA-253) was synthesized and used to formulate oleogels and electrospun nanofibers for 3D printing. The plant-origin dry extract was prepared from a motherwort tincture and valine. The aqueous PEO gels loaded with a synthetic drug (MA-253) containing nanofibers and a valine-modified motherwort extract were prepared and subsequently used in the SSE 3D printing experiments. The homogeneity, viscosity and 3D printability of composite PEO gels were verified. The phytochemical assay of flavonoids in the 3D-printed drug preparations was conducted with the European pharmacopoeia spectrophotometric method.*

Research results. *Three experimental gel formulations loaded with 1,2,3-triazolo-1,4-benzodiazepine nanofibers and a valine-modified motherwort dry extract were developed and tested for the SSE 3D printing applications. The present three gels showed good SSE 3D printability without any significant printing flaws. The SSE 3D-printed lattices prepared from the aqueous PEO gels containing 100 mg/ml of motherwort extract showed the most promising 3D printing performance. The 3D-printed drug preparations were entirely dissolved in purified water (22±2 °C) within 20 minutes, thus suggesting their applicability in oral administration.*

Conclusions. *Novel aqueous PEO gel formulations loaded with nanofibrous 1,2,3-triazolo-1,4-benzodiazepine nanofibers and valine-modified motherwort herb extract are feasible for pharmaceutical SSE 3D printing. The present composite PEO gels enable the preparation of printed oral immediate-release drug delivery systems for new triazolobenzodiazepine derivatives and a drug therapy supportive plant extract*

Keywords: *benzodiazepine derivative, motherwort extract, nanofibers, oleogel, polyethylene oxide, 3D printing*

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1. Introduction

Anxiety disorders (generalized anxiety disorder, panic disorder/agoraphobia, social anxiety disorder, and others) are the most prevalent psychiatric disorders and are associated with a high burden of illness [1]. Anxiety disorders represent a significant medical problem caused by genetic predisposition, environmental stressors, traumatic experiences, and socio-economic factors. High prevalence, chronicity, and comorbidity led the World Health Organization (WHO) to rank anxiety disorders as the ninth (9^{th}) most health-related cause of disability [2]. World widely, anxiety disorders have a profound impact on patients and society. Anxiety disorders account for 3.3 % of the global burden of disease and cost approximately €74 billion in 30 European countries [3]. The estimated lifetime prevalence ranges from 16 % to 34 %, and it reaches 20 % at the end of adolescence [4]. This rate is increasing every year, and consequently, the discovery of new anxiolytic drugs is urgent.

Within the last four years, the COVID-19 pandemic has had wide-ranging impacts on mental health globally, such as social isolation, economic uncertainty and health concerns that may have exacerbated anxiety symptoms for many individuals and provoked mental health issues [5]. *Vice versa,* the presence of mental disorders in anamnesis was a risk factor for severe COVID-19 [6]. For example, in Ukraine, the war the society is currently experiencing, combined with the post-

COVID situation, is the perfect incubator for the growing public health burden of mental disorders [7, 8]. According to the WHO, every fifth person who has been affected by the war is at risk of developing mental illness, and this means that today, there are nearly 8.5 million such people in Ukraine. According to the prediction of the Ministry of Health (MOH) in Ukraine, more than 15 million Ukrainians will need psychological help due to the consequences of the war, and about 3-4 million of these individuals will need medical treatment [7, 9].

The treatment of anxiety disorders typically involves a combination of drug therapy (medication) and psychotherapy. Benzodiazepines and their derivatives are effective for the management of anxiety disorders, and they are widely used in clinical practice. The main limitation of using such drug substances, however, is the prevalent adverse effects [10–12]. Motherwort herb (*Leonurus cardiaca* L.) is one of the most widely used sedative medicinal plants [13, 14]. Rauwald and authors [14] showed the *in-vitro* effects of standardized motherwort herb *L. cardiaca* and *L. japonicus* extracts on the gamma-aminobutyric acid (GABA) site of the GABAA type receptor, which is linked to the transmission of anti-anxiety and antidepressant activity. The neuroprotective effect of motherwort herb on nerve cells in an ischaemic stroke model was investigated due to the presence of leonurine [15, 16]. The motherwort herb tincture can be modified with amino acids due to their ability to form conjugates with phenols, and the present modified tinctures and dry extracts could enhance the pharmacological effects of motherwort herb [13, 17]. Recently, we showed that the motherwort herb dry extract prepared by modifying the motherwort herb tincture with valine amino acid could find uses in pharmaceutical and medicinal applications [13, 18].

The use of new combinations of synthetic and natural compounds in treating diseases could be a promising approach to overcoming the challenges related to conventional single-drug therapy. By combining synthetic and native-origin active ingredients (such as triazolobenzo-diazepines and motherwort extracts in the treatment of anxiety disorders) could provide synergistic effects (an enhanced therapeutic efficacy) and reduce the risk of side effects due to the lower drug dose needed [19, 20]. It is also evident that such combined drug delivery systems (DDSs) are capable of reducing drug tolerance and dependence and consequently improving the safety of the drug treatment. Motherwort herb extracts as an active ingredient also have antioxidant or anti-inflammatory properties [21]. Our strong hypothesis is that by incorporating such natural compounds as a supplement in the DDSs with a synthetic drug, we could improve and provide a comprehensive approach to the treatment of anxiety disorders. The therapeutic effect of triazolobenzodiazepines in the treatment of anxiety disorders has been shown with an animal model in the state-of-the-art literature [22].

New benzodiazepine derivatives, such as recently introduced triazolobenzodiazepines [23], are poorly soluble in water, which greatly limits their use in pharmacotherapy. The electrospun drug-loaded nanofibers were shown to enhance the dissolution and *in-vivo* anxiolytic activity of a poorly water-soluble drug in an animal model [24]. In the present study, we combine triazolobenzodiazepine-loaded nanofibers (in oleogels) and valine-modified motherwort herb extract to prepare a functionalized printing gel. Three-dimensional (3D) printing is an emerging manufacturing technology with several applications in the pharmaceutical dosage form design [25]. Modern 3D printing methods enable the preparation of personalized drug delivery systems (DDSs) based on individual patient needs (i.e., specific doses or combinations of drugs) [26]. In addition, 3D printing enables the fabrication of sophisticated DDSs (such as controlled-release and multi-drug preparations) [27], thus improving the therapeutic efficacy of pharmaceuticals and enhancing patient compliance.

The aim of this study was to develop novel functionalized polyethylene oxide (PEO) gels loaded with 1,2,3-triazolo-1,4-benzodiazepine nanofibers and a valine- modified motherwort herb dry extract for a semi-solid extrusion (SSE) 3D printing. The printing behaviour of such composite gels, and the physicochemical and pharmaceutical properties of final 3D-printed DDSs were studied *in vitro*.

2. Planning (methodology) of research

The study protocol and main steps are presented in Fig. 1.

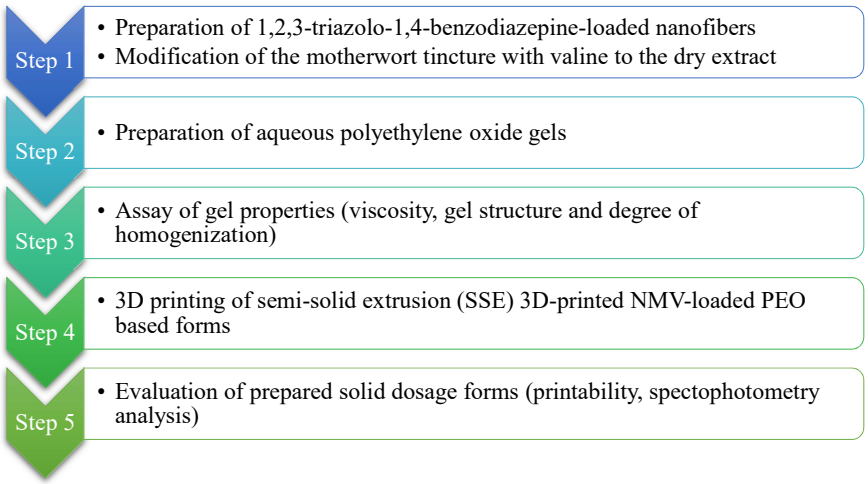


Fig. 1. Study protocol and milestones

3. Materials and methods

Active pharmaceutical ingredient (API).

The 1,2,3-triazolo-1,4-benzodiazepine (MA-253, Fig. 2) was synthesized under the supervision of prof. Chebanov V. A. [28]. The early-stage research results demonstrated this derivative's potential pharmaco-

logical activity [24, 29]. Therefore, the present derivative MA-253 was selected as API in the present study.

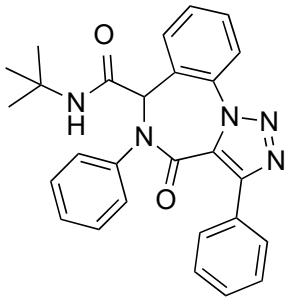


Fig. 2. Chemical structure of 1,2,3-triazolo-1,4-benzodiazepine (a code name MA-253)

MA-253-N-(tert-butyl)-4-oxo-3,5-diphenyl-5,6-dihydro-4H-benzo[f][1,2,3]triazolo[1,5-a][1,4]diazepine-6-carboxamide, which is a gray solid with a melting point >250 °C. Elemental analysis calculated for $C_{27}H_{25}N_5O_2$: C 71.82, H 5.58, N 15.51, found: C 70.60, H 5.64, N 14.78. MS (EI, 70 eV): *m*/*z*=423.05 $[C_{27}H_{25}N_3O_2]^+$.

Preparation of drug-loaded nanofibers by electrospinning.

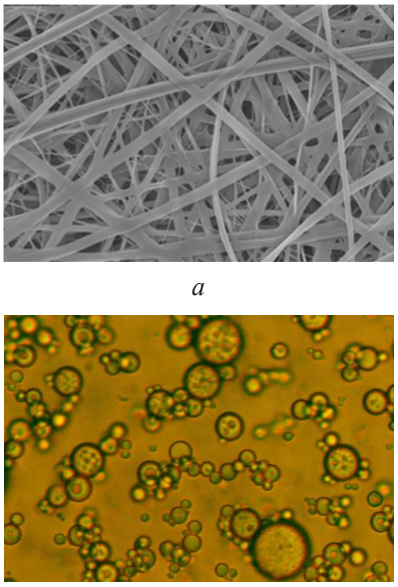
Hydroxypropyl methylcellulose, HPMC (grade 60SH, Shin-Etsu Chemical, Tokyo, Japan) was used as a carrier polymer for generating drug-loaded multilayered nanofibrous mats in an electrospinning (ES) process. For preparing an aqueous HPMC solution, HPMC powder was first dispersed in purified water, and the mixture was allowed to wet and dissolve for overnight under magnetic stirring. Next, acetone (Sigma-Aldrich, St Louis, USA) was added to obtain a 2 % (w/w) HPMC solution, where a volumetric ratio of water to acetone was 6/4.

The solution for ES was prepared in a few steps. The API was first dissolved in acetone at the concentration of 4.75 % (w/w), and the mixture was then stirred for overnight in a magnetic stirrer at an ambient room temperature (22±2 °C) to provide a complete dissolution. Before ES, the HPMC solution was added to this mixture to obtain the final carrier polymer solution for ES (the amount of API was 17.5 % [w/w]).

An ESR200RD robotized ES system (NanoNC, Seoul, Republic of Korea) was used for the ES of nanofibers. The progress of ES was followed visually, and the most stable level for the key process parameters (i.e., high voltage 18.3 kV, flow rate 1.0-2.5 ml/h, and tip-to-collector-distance 17 cm) was selected and controlled to generate a stable Taylor cone. The nanofibers were collected to a stationary roller collector (with a diameter of 90 mm and width of 200 mm) covered with an inert aluminium foil. All experiments were carried out at an ambient room temperature (22±2 °C) and relative humidity, RH (Fig. 3, *a*) [24, 28].

Preparation of oleogels with drug-loaded nanofibers.

The electrospun drug-loaded nanofibers (shown in Fig. 3, *a*) were gently detached from an aluminuim foil. Then, the oleogel was prepared using an equivalent volume of Eumulgin SMO 20 (=surface active agent). The oleogel was homogenized twice in a Retsch Vibrating Mill MM 400 (Retsch GmbH, Haan, Germany) at a frequency of 30/sec for 5 minutes (Fig. 3, *b*).



a

b

Fig. 3. FE-SEM micrographs of the electrospun nanofibers loaded with a new benzodiazepine derivative MA-253 (*a*), and the representative optical microscopy photograph of oleogel (1:1) in an aqueous solution (magnification 200×) (*b*)

Preparation of motherwort herb dry extract with valine.

The dry extract was prepared from a motherwort herb tincture (batch UA/6543/01/01, PJSC Pharmaceutical Factory "VIOLA", Zaporizhzhia, Ukraine). To 270 ml of the tincture, valine (540.0 mg) was added in a triple equimolar amount in relation to the amount of phenolic compounds [21]. The solution was kept at an ambient room temperature (22±2 °C) overnight to obtain the complex substances of phenolic compounds and amino acid (valine). Finally, the solution was evaporated in a vacuum evaporator to a dry extract.

Preparation of aqueous polyethylene oxide (PEO) gels.

PEO (MW approx. 900,000, Sigma-Aldrich, USA), ethanol (Peenviinavabrik, Estonia) and water *R* were used for preparing the aqueous PEO gels loaded with a valine-modified motherwort herb dry extract and 1,2,3-triazolo-1,4-benzodiazepine nanofibers mixed with Eumulgin SMO 20 (polyethylene glycol 40–hydrogenated castor oil, Polysorbate 80) (LOT S721580003, Cognis, France),

The aqueous PEO gel (12 %) was selected as a semisolid platform for the SSE 3D printing of the combination of API-loaded nanofibers and valine-modified motherwort herb dry extract. For preparing aqueous PEO gels, PEO (1.2 g) was first dissolved in distilled water (volume of 10 ml minus the volume of ethanol for dissolution of NMV). The ethanolic mixture of NMV (0.5 g, 1.0 g or 1.5 g of NMV dissolved in 1 ml of ethanol) and Eumulgin SMO 20 (at the surface-active agent-PEO ratio of 1:2) were then added to the PEO solution. Finally, the present mixture was homogenised and

kept at an ambient room temperature (22±2 °C) for at least 13–15 hours to form a viscous gel [30, 31].

Characterisation of aqueous PEO gels.

The viscosity of gels was investigated with a Physica MCR 101 rheometer (Anton Paar, Austria) using a cone-plate geometry at an ambient room temperature (22±2 °C). The viscosity measurements were performed using a rotational shear test at 0.060 1/s. The gel structure and the degree of homogenization were studied with an optical light microscope (Magtex-T Dual Illum., Medline Scientific, United Kingdom) equipped with a digital camera (Industrial Digital Camera UCMOS09000KPB (9.0 MP 1/2.4" APTNA CMOS sensor).

Semi-solid extrusion (SSE) 3D printing of NMV-loaded aqueous PEO gels.

The PEO gels loaded with NMV were printed using a bench-top SSE 3D printing system (System 30 M, Hyrel 3D, USA). The printing head was composed of a steel syringe featuring a plunger linked to a stepper motor, where the motor's movement either raises or lowers the plunger, facilitating the dispensing of the content within the syringe. A printing nozzle system was a blunt needle (Gauge, 21G) connected to the syringe. The printing head was applied without heating during a 3D printing. Throughout a SSE 3D printing process, a printing head moved at a predetermined speed along the *X-Y* axis (referred to as the printing speed) and extruded the printing material through a nozzle system at a specified speed (referred to as the extrusion speed) onto a printing plate. The temperature of the printing plate was maintained at 30 °C. After each layer was printed, a printing plate underwent a predetermined descent by a specified distance, known as the layer height. This process enabled a printing head to generate another material layer on the surface of a previously printed layer. The software (Repetrel, Rev3.083_K, Hyrel 3D, USA) used in a SSE 3D printer controlled the temperature of a printing head and plate, the movement speed of the printing head, gel extrusion rate, and other relevant settings. The printing head operated at a speed of 0.5 mm/s. The model lattices comprised a total of eight (8) printed layers, while the round-shaped disc preparations were printed to have a total of five (5) layers [32].

For verifying a 3D printing quality, a model 4×4 grid lattice was used [30]. The square-shaped 3D lattice had the final dimensions of 30×30×0.5 mm. The assessment of 3D printability relied on the measurements of the printed lattice's weight and area. A comparison was made between the theoretical surface area of a model square-shaped 3D lattice (324 mm²) and the corresponding areas of the experimentally 3D-printed lattices [18, 30]. A round-shaped disc preparation (20 mm in diameter) was designed by using a FreeCAD software (vers. 0.19/release date 2021).

The SSE 3D-printed PEO lattices and round-shaped disc preparations were weighed with an analytical scale (Scaltec SBC 33, Scaltec, Germany) and photographed. Image analysis was conducted on the photographs using ImageJ software (version 1.51k) from the National Institute of Health, USA. For the 3D-printed lattices, the experimental surface area value was compared with the corresponding theoretical value of the designed lattice. The surface characteristics of the 3D-printed round-shaped discs were studied with an optical light microscope (Magtex-T Dual Illum., Medline Scientific, United Kingdom) equipped with a digital camera (Industrial Digital Camera UCMOS09000KPB, 9.0 MP 1/2.4" APTINA CMOS sensor).

Determination of flavonoids.

An established spectrophotometric method (described in the Monograph "*Leonurus tincture*" of the State Pharmacopeia of Ukraine [33]) was used for the quantitative determination of flavonoids in the extract and 3D-printed DDSs in terms of a dry residue and hyperoside. The optical density of solutions was measured with a Specol 1500 spectrophotometer (Thermo Fisher Scientific, Basel, Switzerland). The total flavonoid content was determined in terms of hyperoside (at a wavelength of 425 nm) after the formation of the complex with aluminium chloride [34, 35].

Statistical analysis.

Data was statistically assessed using a MS Excel software (Microsoft Excel 2016, version 16.0, Microsoft Corporation, USA) [34].

4. Results

Physical appearance and viscosity.

Table 1 shows the composition of aqueous PEO gels loaded with 1,2,3-triazolo-1,4-benzodiazepine nanofibers and a valine-modified motherwort dry extract designed for SSE 3D printing. The valine-modified motherwort herb dry extract was first dissolved in a small amount of ethanol. Then, the emulsion with drug-loaded nanofibers was added and thoroughly mixed with an ethanolic mixture. The present solution was used for preparing a final aqueous PEO gel. The functionalized PEO gels were homogeneous and moderately viscous brownish masses with a specific smell.

Table 1

Composition of aqueous polyethylene oxide (PEO) gels loaded with 1,2,3-triazolo-1,4-benzodiazepine nanofibers and a valine-modified motherwort herb dry extract

Sample	Modified motherwort extract (g)	Nanofibers mixed with oleogel (g)	Eumulgin SMO 20 (g)	PEO (g)	Ethanol (ml)	Water (ml)
1	0.5	0.04	0.560	1.196	1.00	9.00
2	1.0	0.08	0.498	1.198	1.00	9.00
3	1.5	0.12	0.476	1.198	1.00	9.00

The assessment of viscosity is crucial with the semisolids and gels intended for 3D-printing since it is necessary to adjust the fluid mechanics to align with the movements of a printer platform to ensure the highest quality of 3D-printed objects [36]. Table 2 shows the results of the viscosity measurements for the three functionalized PEO gels studied. The viscosity measurements were conducted at room temperature (22±2 °C).

The physical appearance and homogeneity of functionalized PEO gels were studied with an optical light microscope equipped with a digital camera. The three PEO gels loaded with 1,2,3-triazolo-1,4-benzodiazepine nanofibers and a valine-modified motherwort herb dry extract presented a yellow-to-brown colour and a moderate-to-good degree of homogeneity (Fig. 4).

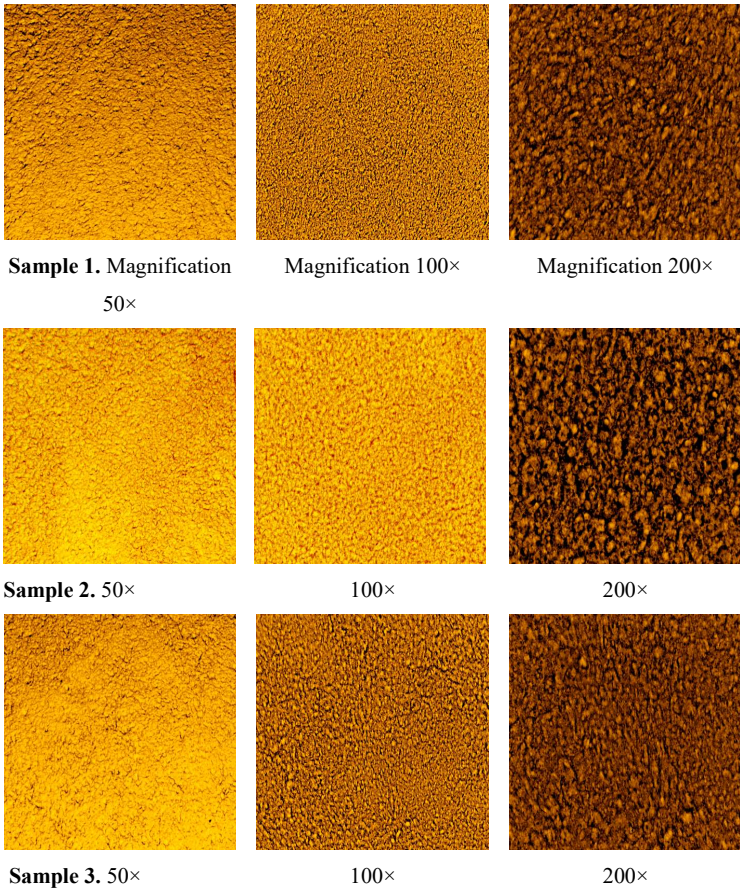


Fig. 4. Optical light microscopy images of aqueous polyethylene oxide (PEO) gels loaded with 1,2,3-triazolo-1,4-benzodiazepine nanofibers and a valine-modified motherwort herb dry extract. Magnification 50×, 100× and 200×. Reference is also made to Table 1

Table 2

The viscosity of aqueous polyethylene oxide (PEO) gels loaded with 1,2,3-triazolo-1,4-benzodiazepine nanofibers and a valine-modified motherwort herb dry extract. Reference is also made to Table 1

Sample	Viscosity, cP (speed 0.03 RPM, shear rate 0.060 1/s, temperature 22±2 °C, *n*=3)
1	147200±16633
2	177000±17985
3	259333±9734

SSE 3D printability of functionalized PEO gels. Viidik et al. [37] identified and optimized the key SSE 3D printing parameters for aqueous PEO gels. In the present study, we relied on the findings of Viidik and co-authors in selecting the printing conditions for our functionalized PEO gels. For SSE 3D printing of PEO gels, the temperature of a printing head (with a blunt needle) and a printing plate was maintained at 30 °C. The printing head speed was set at 0.5 mm/s. The printing performance of functionalized PEO gels was investigated by printing standard-sized square-shaped 3D lattices with constant dimensions of 30×30×0.5 mm and then assessing the quality of the final printed DDSs [38]. Table 3 summarizes the results of 3D printing experiments and quality assessment (i.e., the weight and surface area of 3D-printed lattices).

To assess the printing quality of final DDSs, both lattices and round-shaped discs were printed from the functionalized PEO gels. The photographs of such printed lattices and round-shaped discs are shown in Fig. 5, 6, respectively. The lattices were printed at the printing head speed of 0.5 mm/s, and a total of eight layers were formed (Fig. 5).

The SSE 3D-printed lattices exhibited a moderate-to-good printing quality and good reproducibility, thus suggesting that the present functionalized PEO gels are feasible for their intended use.

Fig. 6 shows the photographs of the SSE 3D-printed round-shaped discs intended for oral administration (Sample 1 as an example formulation). These preparations consisted of a total of five printed gel layers, and the discs were 3D printed at a constant printing head speed of 0.5 mm/s. The average weight of SSE 3D-printed discs (Sample 1) was 110.0±2.2 mg.

Table 3

Weight and surface area of the semi-solid extrusion (SSE) 3D-printed lattices (*n*=3). The printed lattices were prepared from the functionalized polyethylene oxide (PEO) gels loaded with 1,2,3-triazolo-1,4-benzodiazepine nanofibers and a valine-modified motherwort herb dry extract. Reference is also made to Table 1

Sample	Weight, mg	Area (*S*), mm^2	$S_{practical}/S_{theoretical}$
1	211.0±7.1	390.2±20.7	1.20
2	241.1±10.5	347.6±19.1	1.07
3	307.2±5.7	412.5±25.4	1.27

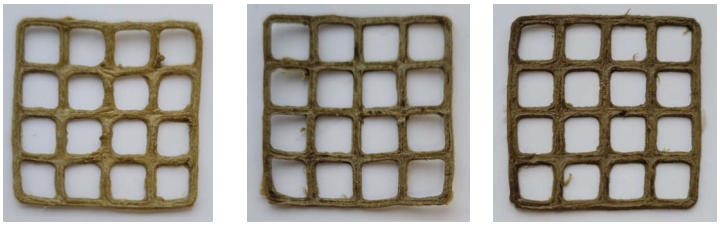


Fig. 5. The semi-solid extrusion (SSE) 3D-printed lattices (Samples 1–3). The printed lattices were prepared from the functionalized polyethylene oxide (PEO) gels loaded with 1,2,3-triazolo-1,4-benzodiazepine nanofibers and a valine-modified motherwort herb dry extract. Reference is also made to Table 1

Fig. 6. The semi-solid extrusion (SSE) 3D-printed round-shaped discs (n=3). The printed discs were prepared from the functionalized polyethylene oxide (PEO) gel (Sample 1) loaded with 1,2,3-triazolo-1,4-benzodiazepine nanofibers and a valine-modified motherwort herb dry extract. Reference is also made to Table 1

In-vitro dissolution of SSE 3D-printed lattices.

The *in-vitro* dissolution test of SSE 3D-printed lattices was carried out in purified water (without and with stirring) at an ambient room temperature (22±2 °C). The dissolution behaviour of printed lattices was visually observed for 30 minutes. The SSE 3D-printed lattices were fully disintegrated/dissolved approximately within 20 minutes. This suggests the feasibility of the present 3D-printed DDSs for oral immediate-release administration.

Assay of flavonoids by spectrophotometry.

The flavonoid content of SSE 3D-printed drug preparations (Samples 1–3) was determined by the established spectrophotometric method described in the European Pharmacopoeia [18, 33, 34]. The contents of flavonoids in 3D-printed DDSs (Samples 1–3) were 0.30±0.09 %, 0.39±0.02 % and 0.46±0.03 %, respectively.

5. Discussion

Contemporary 3D printing technologies provide a versatile platform for preparing highly innovative personalized pharmaceutical dosage forms [26]. Moreover, 3D printing is a valuable technique especially for poorly water-soluble drugs, such as benzodiazepine derivatives, to overcome the challenges related to their formulation, and to enhance their oral bioavailability and therapeutic efficacy [39, 40]. Pharmaceutical SSE 3D printing enables also to combine two or more active agents in the same final DDSs without the risk of physicochemical incompatibility between such compounds.

In the present study, the functionalized PEO gels developed for SSE 3D printing were viscous semisolids with varying degree of a yellow-to-brown-to-greenish colour. The variation in colour was due to the presence of motherwort herb dry extract at different concentrations in the gels. The functionalized PEO gels loaded with 1,2,3-triazolo-1,4-benzodiazepine nanofibers and a valine-modified motherwort herb dry extract presented quite a homogeneous gel structure, which was confirmed by means of optical light microscopy (Fig. 3). No significant sediments nor clumps were observed, thus suggesting the applicability of such aqueous gels in SSE 3D printing.

In our previous study, we found that with (non-functionalized) PEO gels, printing at least eight (8) layers is sufficient for generating the final 3D-printed lattices of high quality [30]. Therefore, we also selected eight layers in the present study for the SSE 3D printing of functionalized PEO gels. The SSE 3D printing performance and quality of functionalized PEO gels were evaluated by the visual inspection of the layout of the corresponding 3D-printed lattices and based on the calculations of an $S_{practical}/S_{theoretical}$ ratio. All functionalized PEO gels studied were readily printable in SSE 3D printing, and the corresponding final printed lattices showed a good printing quality with high reproducibility. The best 3D-printing performance (i.e., the lowest $S_{practical}/S_{theoretical}$ ratio of the lattice) was found with the functionalized PEO gel containing motherwort herb dry extract 100 mg/ml (Sample 2 in Table 1). The present findings indicate the feasibility and reliability of an SSE 3D printing process in preparing the oral DDSs loaded with 1,2,3-triazolo-1,4-benzodiazepine nanofibers and a valine-modified motherwort herb dry extract.

The *in-vitro* dissolution test of SSE 3D-printed DDSs was carried out by visually determining the disintegration/dissolution time for the printed preparations (round-shaped discs) in purified water (22±2 °C). According to the European Pharmacopoeia, film-coated tablets for oral administration should be disintegrated in purified water at 37 °C within 30 minutes [34]. The present SSE 3D-printed DDSs (round-shaped discs) prepared from the functionalized PEO gel disintegrated/dissolved completely within 20 minutes, thus complying with the specification of the European Pharmacopoeia. Since our test protocol was not identical as described in the European Pharmacopoeia, the present results on the disintegration/dissolution of 3D-printed DDSs can be considered as only indicative. These preliminary results, suggest that the 3D-printed DDSs developed here could be feasible for oral administration. Recently, Wang et al. (2022) reported the use of plant-based materials in extrusion-based food 3D printing and discussed about the potential uses of such printed food [41].

We found that the spectrophotometry method used in the present study is feasible for the assay of flavonoids in the SSE 3D-printed drug preparations. The present spectrophotometry method can also be used for the standardization of valine-modified motherwort herb dry extracts for pharmaceutical formulation development [42–44].

Study limitations. To date, no standardized analytical methods have been developed for the present new benzodiazepine derivatives. Therefore, it is impossible to determine the release of such benzodiazepines from the drug-loaded nanofibers in functionalized PEO gel and 3D-printed drug preparations. The pharmacological activity of the present combination of triazolo-1,4-benzodiazepine derivatives and motherwort herb extract material has been not studied and known. Therefore, the potential synergistic therapeutic effect of the present new benzodiazepine derivative and plant-origin material is only based on a theoretical hypothesis.

The prospects for further research. The *in-vitro* disintegration/dissolution of the SSE 3D-printed drug preparations will be studied using an established pharmacopoeia method (European Pharmacopoeia). Our forthcoming studies will investigate and verify the pharmacological activity and standardization of the present SSE 3D-printed drug preparations.

6. Conclusions

The new functionalized PEO gel formulations combining 1,2,3-triazolo-1,4-benzodiazepine nanofibers and a valine-modified motherwort herb dry extract were developed and optimized for pharmaceutical SSE 3D printing. The results show that the drug-loaded nanofibers can be homogeneously mixed with the motherwort herb dry extract in the PEO gels. The functionalized PEO gel containing motherwort herb dry extract 100 mg/ml presents the most promising SSE 3D printing performance. The SSE 3D-printed drug preparations developed in the present study are applicable for oral immediate-release administration. Further studies are needed to verify the therapeutic efficacy and safety of the present composite 3D-printed DDSs of new 1,2,3-triazolo-1,4-benzodiazepine derivative and a valine-modified motherwort herb dry extract in the drug treatment of anxiety.

Conflicts of interest

The authors declare that they have no conflict of interest concerning this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this paper.

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Data availability

Data will be made available on request.

Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies when creating the current work.

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