

Controlled Release of Bi-Layered Malvidin Tablets using 3D Printing Techniques

Abstract Malvidin belongs to the class of anthocyanidin, a pigment compound present in fruits and vegetables like the colored berries, flowers, and vegetables which have pigments on it and it is available commercially as malvidin chloride. Malvidin is known to possess many medicinal characteristics like anti-microbial, anti-diabetic, anti-inflammatory, anti-obesity, and anti-cancer. In this research paper, a 3D printing technique is used which evolves a 3D printer based on desktop that extrudes tablets comprising the active drug which here is malvidin our main ingredient and the other excipients which are used as binders and disintegrants. Methods which are adapted here for the formulation of 3D printed tablet make the tablets appropriate for immediate and sustained release with its definite physical and mechanical properties like hardness, friability, and weight. Tablets that are extruded by the 3D printer are controlled release bi-layer tablets. Due to involvement of 3D printer, printing cost for the bi-layered tablets found very low that makes our method as cost efficient.

Keywords Anthocyanidin, bi-layer tablets, malvidin, 3D printer.

INTRODUCTION

Malvidin is an anthocyanidin which can be obtained from pigmented fruits, vegetables and flowers. Anthocyanidin is a natural pigment which is the precursor anthocyanins which gives us the blue-red colours of fruits and flowers [1]. Malvidin is plant pigment and is biologically active. Cell signaling can be studied as an inhibitor of 3', 5'-cyclic adenosine monophosphate (cAMP) phosphodiesterases (PDE) and some cGMP-specific phosphodiesterases of o-methylated anthocyanidin. Malvidin can also be used as reference material for anthocyanidin glycosides. The structural formula of the malvidin is shown in Fig. 1.

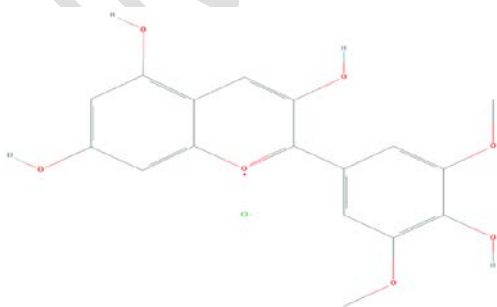


Fig. 1: Chemical structure of malvidin [2].

Solubility of Malvidin:

In our research we found that anthocyanins are soluble in water but for the exception, malvidin is soluble slightly in water. To increase its solubility, we found a method which increases the solubility of a flavonol component or of a flavonol-containing composition in water [3].

Besides its color properties, anthocyanins have various health attributes such as anti-inflammatory effects, anti-carcinogenic activity, reduced risk of coronary disease and improved cognitive behavior [4], [5]. The anthocyanin, just like pH paper, appears to be red in color when present in acidic condition and blue in color when present in alkaline. In terms of its stability, anthocyanins depend on temperature, light, pH, and its structure [6].

Flavonoids are found in fruits, vegetables, grains, bark, roots, stems, flowers, tea and wine and are the collection of natural substances having variable phenolic structures [7]. The different types of flavonoids present can be seen in the Fig. 2 with their sub classes.

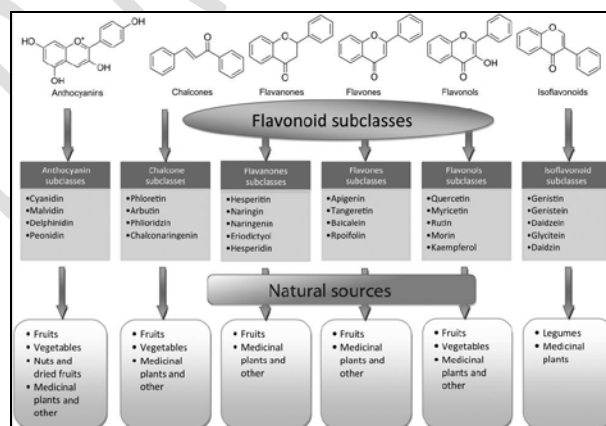


Fig. 2: Different types of flavonoid and its subclasses [7].

Fruits, tubers, flowers are prominent sources of blue, red, purple pigments which constitutes anthocyanins.

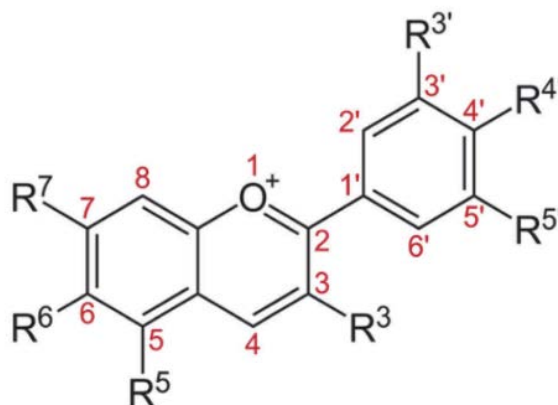


Fig. 3: Basic structure of Anthocyanins [8].

In plants, anthocyanins are very beneficial as the pigments are derived from the plant sources such as fruits, flowers, vegetables, and are commonly used as food colorants. In addition to these particular anthocyanin applications, medicinal properties such as anti-inflammatory, anti-cancer, anti-microbial, anti-diabetic, anti-obesity and cardiovascular disease prevention are also found [9]. Different forms of anthocyanins could be present in plants a few of them with their structures could be seen presented below in Fig. 4 whereas the basic chemical structure of anthocyanins can be seen in Fig. 3.

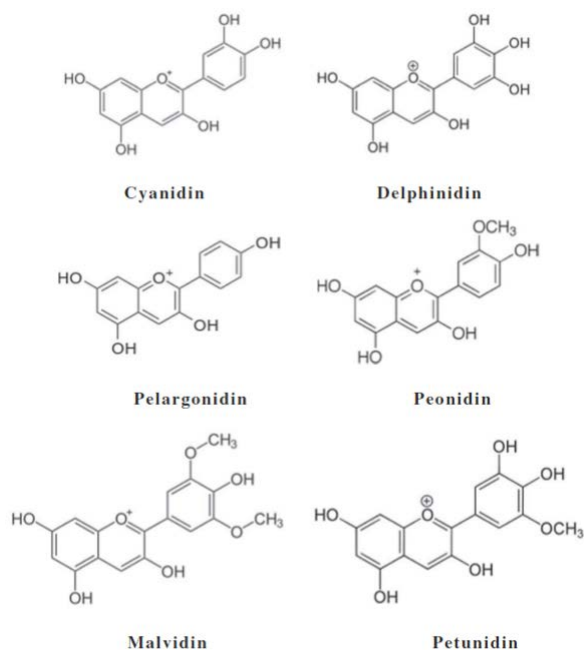


Fig. 4: Major sources of anthocyanidins found in plants [8].

The soluble form of anthocyanin pigments:

As described earlier regarding anthocyanin pigments, which appears blue or purple in alkaline medium and red in acidic medium, and are derived from flowers and fruits, red and purple berries and even anthocyanins found as flavylium ions in grapes and wines. Water serves as an anthocyanin extraction medium, since water serves as a solution medium for anthocyanin because anthocyanin can be dissolved in both the water and the organic solvent [8]. The flavylium ions structure can be seen in Fig. 5.

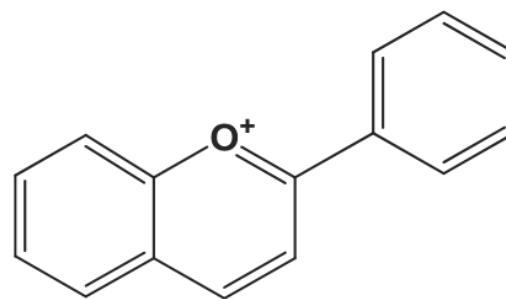


Fig. 5: Structure of flavylium ion in two-dimensional view [8].

Plant used in the malvidin extracts:

Pigmented fruits, vegetables and flowers, tubers are prominent sources of blue, purple pigments which constitute malvidin. Blueberries or highbush blueberries or *Vaccinium corymbosum* (Family: Ericaceae) are major source of malvidin and traditionally grown for thousands of years by the Native Americans. Blueberry, a deciduous shrub, grows up-to the height 6–12 feet. In present study, blueberry extract powder [10], a high malvidin containing powder is used to produce tablets from 3D printing techniques. Blueberry extract powder promotes cardiovascular health, improves cognitive functions and is natural antioxidant.

There have been many difficulties faced by traditional methods during tablet formulation, such as the error caused by the formulation, the milling process often causes fines resulting in spots, hardness, etc., some compressions of failure and some problems of ejection which may result in sometimes too dry or too wet tablets. By using 3D printer these all issues can be avoided as the 3D printer offers specific formulation such that it can be produced precisely not so wet and not so dry tablet. Even 3D printer minimizes human errors.

The purpose of this paper is to design effective tablets without affecting the therapeutic properties of malvidin utilizing a relatively cheap process consisting of a 3D printer. With Food and Drug Administration authorizing the first 3D printed drug in 2015, this technology was used to advance the pharmaceutical industry field that ultimately boosts healthcare sectors. The usage related to 3D printers is highly abundant in the branches of technology, aerospace, construction, automobiles, dentistry, robotics, etc. but their full potential is still yet to be uncovered in the field of pharmacy [11]. In the pharmaceutical industries, between the years 1993-2003, the boost of powdered tablets created using 3D printer led to the oral dosage forms. After that the industry is continuing to advance [12].

The most used form of drug administration is the consumption of the tablets that can be administered orally. Usually tablets are prepared by single or multiple compressions [13][14][15]. Traditionally for producing the powdered tablets, was to compress the tablets by grinding, mixing and granulation systems, making tablets that could

be dry or wet as per their production method [16][17]. The tablets are produced around the world in large-scale factories with massive plants to be processed under stringent regulations to ensure the safety and correct dosage of the medication in the tablets [18]. The works relevant to the previous experiments demonstrate the promise of printed medicines. The inkjet printer can also be used to containing drugs such as paracetamol, theophylline and caffeine [19]. However, the medication was shown to deposit just few micrograms. A dynamic, multi-step 3D printing process was imported to manufacture the solid dosage forms [12] [20]. So to solve all these high cost tablet formulations and production processes, the use of 3D printed tablet costs very little relative to these large pharmaceutical companies to produce. Using the 3D printer (a 3D printer mounted on a desktop) and its software helps to accomplish this goal. Using 3D printers has enabled to create viable 3D printed tablets that can be released immediately and sustainably.

RESEARCH QUESTIONS

- How can bi-layer tablets be produced with 3D printer using a natural compound?
- Will there be a method available which can be developed in a cost-effective manner with the development of bi-layer tablets?

METHODOLOGY

Design of the experiment:

The design of the experiment follows certain steps which are as follows:

Malvidin paste Preparation steps:

1. Intended for immediate release layer: The malvidin powder along with required excipients intended for immediate release layer that have been utilized here are microcrystalline cellulose (MCC) and sodium carboxymethyl cellulose (SSC) that were mixed for minimum 30 minutes of time interval. HPMC 2906 (1% w/v) was used as a binder in powder blend. After that HPMC 2906 gel (previously adjusted volume) mixed till the paste turns homogenous and no separation and aggregates were observed.
2. Intended for sustained release layer: The malvidin powder along with required excipients intended for sustained release layer that were used includes HPMC2208 (with various percentage) and Poly Acrylic Acid (PAA) which were mixed for minimum 30 minutes. Whereas HPMC2208 (1% w/v) was used as the malvidin powder's binder that holds all other ingredients collectively which form

the paste.

Each prepared pastes were packed individually into different syringe tool, placed on the 3D printer and then malvidin bi-layered tablets were extruded from the 1.2 mm nozzles by using software (FabStudio) in the 3D printer.

Hydroxypropyl methylcellulose gel preparation:

Hydroxypropyl Methylcellulose or HPMC have different level of viscosity grades were being used for immediate release layer [HPMC 2906 (1% w/v)] and for the sustained release layer [HPMC2208 (1% w/v)].

HPMC 2906 (1% w/v) preparation intended in gel form: HPMC 2906 powder (one-gram) was taken and added to the hot water (about 30 ml) and vigorously stirred for about 25-30 minutes. Then stirring was conducted in such a manner as to blend it properly and for creating a proper dispersion. Ice cubes of about seventy grams were then applied to it and then stirred thoroughly which increases the solubility of HPMC material in water. After that this obtained gel (gel-alike mixture) was put for refrigeration where it can be contained for at least minimum of 24 hours in order to release air bubbles and to create a strong concentration of homogeneous gel [21].

HPMC2208 (1% w/v) preparation intended in gel form: HPMC2208 powder was taken of one-gram quantity and added to the hot water (about 30 ml) and vigorously stirred for about 25-30 minutes. Then stirring is conducted in such a manner as to blend it properly and creates proper dispersion. Ice cubes of about seventy grams is then applied to it and then stirred thoroughly which increases the solubility of HPMC material in water. After that this obtained gel (gel-alike mixture) is put for refrigeration where it can be contained for at least minimum of 24 hours in order to release air bubbles and to create a strong concentration of homogeneous gel [21].

Sample Materials:

1. Malvidin chloride powder (Appearance: Reddish brown to black powder) the active component of the tablet (from Enzo Life Sciences). (Also it can be utilized from Blueberry extract powder (from herbadiet), (bluish-purple coloured powder)).
2. Hydroxypropyl Methylcellulose (HPMC)
 - HPMC 2906 (Hypromellose, from Sigma-Aldrich)
 - HPMC2208 (Methocel K100M Premium, from Colorcon)
3. Microcrystalline cellulose(MCC from Pharmacol of no. 102)
4. Sodium Carboxymethyl Cellulose (SCC) (from Sigma-Aldrich)
5. Poly Acrylic Acid (PAA, carbopol of no. 974P NF, from Surfachem Group)
6. Trisodium Phosphate Dodecahydrate (from

Sigma-Aldrich)

Instrument:

- 3D Printer (on Desk-top) with tray having movement in x-y axis and was used to get the 3D printed tablets. Whereas, two nozzles movement in z-axis was used to extrude malvidin bi-layered tablets.
- United States Pharmacopeial (USP) Convention Type-I apparatus was used for in-vitro release (Dissolution Tester- by Erweka of no. Dt600) which creates a low pH or acidic medium that represents the human stomach.
- C50 Hardness tester, by I Holland Ltd.
- Friability tester E-1851, Erweka.

Data collection:

Table 1: Different constituent's composition for immediate release

Constituent's composition	(Percentage w/w) per Immediate Release layer
Malvidin	77
MCC PH 102 (disintegrant)	11.5
SSG type A (disintegrant)	9
HPMC 2906 (binder)	2.5

Table 2: Different constituent's composition for sustained release

Constituent's composition	HPMC2208 combined with the active drug ingredient			
	6% w/w	8% w/w	10% w/w	14% w/w
Malvidin	88	86	84	80
HPMC2208 (hydrophilic matrix)	6	8	10	14
Poly Acrylic Acid (hydrophilic matrix)	3	3	3	3
HPMC2208 (binder)	3	3	3	3

Data analysis:

Referring to Table 1 & 2, the forms of disintegrants used to research the immediate release functionality were sodium carboxymethyl cellulose (SSC) and microcrystalline cellulose (MCC). Sustained release capability was tested by using hydrophilic matrix; poly acrylic acid (PAA) and HPMC2208 at four separate percentages of HPMC2208 [(6% w/w), (8% w/w), (10% w/w), (14% w/w)].

For the better clarification, a desktop-based 3D printer was used to extrude bi-layered tablets of malvidin (using FabStudio software). Malvidin bi-layered tablets containing malvidin (as an active drug) was printed using a 3D printer which formulates numerous sustained release tablets.

Examining in-vitro drug release: A Type-I apparatus of the United States Pharmacopeial (USP) Convention with the 3D printed tablets used for in-vitro release for 2 hours in acidic medium ((representing the stomach)) at 50rpm.

Thereafter, a solution of trisodium phosphate dodecahydrate with a concentration of 0.2 M was placed which raises the pH level to about 6.8 and will reflect the gastrointestinal fluid.

Five 3D printed tablets were taken and placed in the acidic medium of 0.1M HCl of quantity 675ml. Then about 5 ml samples from the acidic solution were taken out at 0.25, 0.5, 01, and 02 hour's duration [22]. Then 0.2 M trisodium phosphate dodecahydrate solution was placed in it after two hours of time have been achieved and it raises the solution's pH to 6.8 [22]. The pH can be reversed by addition of few drops of HCl solution of 0.2M concentration in it. Afterwards 5ml volume of sample at two, four, six, eight, and ten and twelve hours of intervals were taken out consequently. And then the visible UV-spectrophotometer used to evaluate 1ml of solution that is taken from each 5ml sample solution after diluting it with required dissolution medium (taken 9ml in quantity and at a temperature of $98.6^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ of temperature).

Physical characterization of 3D printed tablets:

Friability: Randomly 15-20 numbers 3D printed tablets are taken and placed on a sieve where if any loose dust present is dusted using a soft brush. Subsequently weight of each tablet is taken and is placed on friability tester where it gets rotated on a constant rotational speed (25rpm) for 5 minutes. Now these tablets were placed on a sieve and were dusted again using a brush and then is weighted again and the loss percentage is calculated [23] [24] [25].

Weight: Percentage of differences in the weight of twenty individual tablets were calculated and further related with their average [23] [24].

Hardness: The designed 3D tablets should be delicate, and it should be easy to disintegrate and release the substance, although it should be strong enough to avoid it from being quickly broken during its transport and storage. Around Five 3-dimensional printed tablets are taken and were tested on a tester machine for hardness. (C50, hardness tester, by I Holland)[23][24][26].

RESULTS & DISCUSSION

Dissolution of 3D printed tablets:

The initial burst release of the active drug occurred (less than 20% in 0.5 hours) by the immediate release layer of the tablet designed. Because of the disintegrants added in the formulation of the tablets, the immediate release was observed good, as the high amount of the active drug was released. In two hours of time interval, the initial release of the active drug with HPMC2208 in the 6% w/w and the 8% w/w was found to be high (>70%) as compared to the 10% w/w and the 14% w/w (around 55% or more). This happened due to some small channels which are found on the surface

side of the 3D printed tablets. The release of the active drug with 14% w/w HPMC2208 was found to be consistent and when the amount of HPMC2208 increased than the active drug's release decreases. HPMC increase leads to increased water uptake, better wettability and greater swelling of the gel barrier formulation and the hydrophilic matrix which are consistent to the reduction which is observed in the drug release rate with greater amount of HPMC2208[25]. The dissolution of the 3D printed tablets can be seen in Fig. 6.

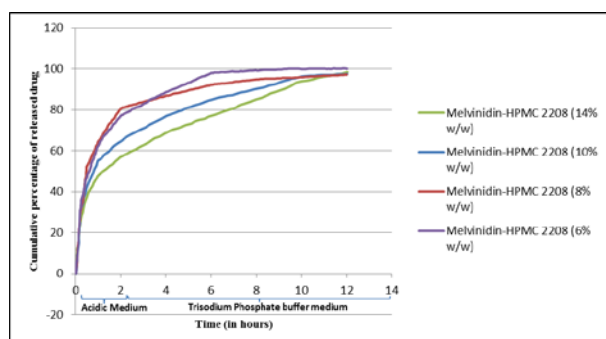


Fig. 6: Malvidin with HPMC2208 [(6% w/w), (8% w/w), (10% w/w), (14% w/w)] and their dissolution profiles is shown in the graph.

3D printed tablets' mechanical properties:

3D printed tablets' mechanical properties were tested for the criteria- hardness, friability, and the weight variation according to USP specifications [26]. During preparation, the overall mass can differ marginally and the weights of all the printed tablets can range from 650 mg to around 750 mg, which is a range for many industrial two-layer tablets. HPMC2208 6% w/w 3D printed tablet with the active drug was found to be having the highest variation compared with among other concentrations. It can be observed that the 3D printed tablets were treated and processed without loss in its structural integrity. The factor friability has variation due to the low percentage of binder and low viscosity grade of the HPMC2900 1% w/w in the immediate layer which is also a binding agent [27]. The friability of HPMC2800 14% w/w with the active drug was found optimum for the further applications.

Drug release mechanism of the printed drug:

Under acidic conditions, the drug released in the first two hours of the cycle and under the buffer conditions released in 2 hours to 12 hours. [25][28][29].

Evolved method for making bi-layered 3D printed malvidin tablets in the present research produces tablets that are sturdy enough to not be broken into pieces during their storage and transport and are fragile enough to quickly disintegrate when consumed. It is possible to see the drug release kinetics which indicates that the immediate release can be obtained between 2-14 hours from the time intervals of 0-2 hours, and the continuous release of the medication. Adapted 3D printing technology often allows the tablet

cost-effective.

CONCLUSION

Malvidin bi-layer tablets and their complex composition have been extruded using a 3D printer and is a cost-effective method. The 3D printing technologies are increasingly emerging and in the field of pharmaceutical sciences which will allow the complicated procedure to be carried out in a very cost-effective and time-efficient manner which will increase output volumes and make tablet costs economical. 3D printer may be used for the development of modern bi-layer or multi-layer medications and new tablet designs. In a making a healthier country, 3D printing of extruded tablets will in some way contribute.

The bi-layered tablets produced were created using separate analyzes and methods such that the tablets set properties were not affected. The immediate release and the sustained release processes were examined separately. When the 3D printed tablets with specified time periods for the immediate and sustained release were formulated, the ultimate stage of the study completes without altering the tablet's defined mechanical properties. The approach offers a fully pure tablet to support certain individuals who are vegans and as such it may not have any adverse effect but it can be used as a substitute.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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