International Research Journal on Advanced Engineering and Management
https://goldncloudpublications.com
https://doi.org/10.47392/IRJAEM.2025.0182

e ISSN: 2584-2854
Volume: 03
Issue:04 April 2025
Page No: 1116-1121

Repurposing Beetroot Waste for Sustainable Production of Bioink for 3-D Printing Applications

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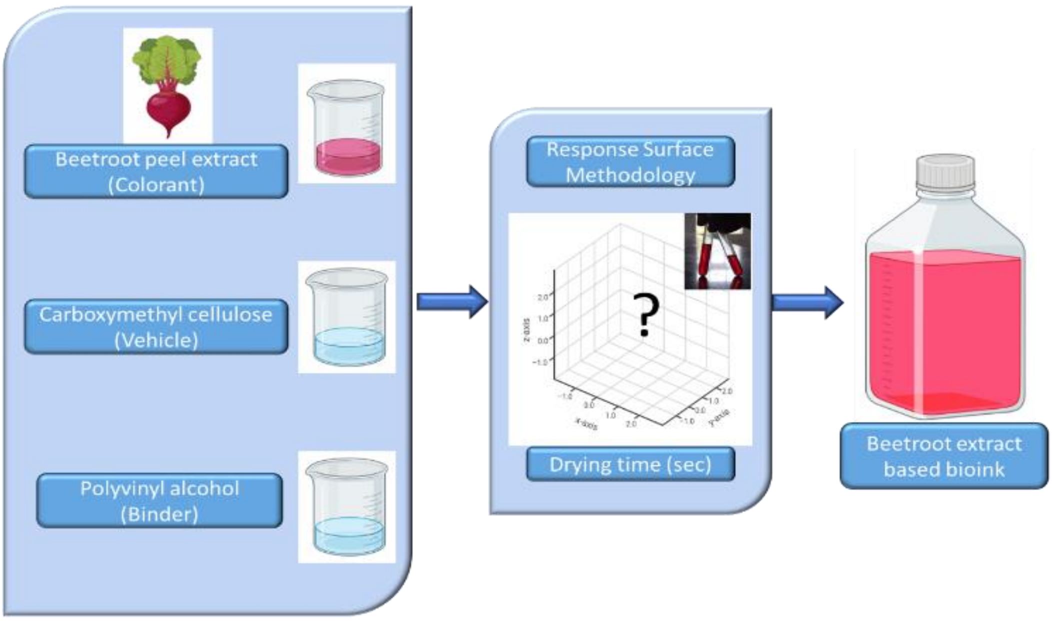
Abstract

The development of bioink from beetroot waste offers a sustainable and eco-friendly alternative to harmful chemicals containing traditional inks. Chemical inks like congo red, methylene blue, crystal violet and other synthetic dyes are mutagenic and carcinogenic in nature. Bioink offers a sustainable solution as it involves utilizing biowaste as the primary raw material for its production, reducing waste and promoting eco-friendliness. This study used the approach of central composite design of response surface methodology for optimising the concentration of components like carboxymethyl cellulose (CMC), polyvinyl alcohol (PVA) and beetroot extract for the formulation of bioink. By utilising natural pigments like betalains and anthocyanin, bioink reduces the risks associated with mutagenic and carcinogenic substances. This innovative approach promotes waste reduction, recycling, and the creation of less harmful products. The study's use of response surface methodology to optimise bioink formulation demonstrates the power of mathematical modeling in improving bioink quality. An approach such as this results in harmless products for both users and manufacturers, contributing to a more environmentally responsible industry.

Keywords: *Bioink, Beetroot, RSM, Drying Time, Carboxymethyl Cellulose, Polyvinyl Alcohol.*

Graphical Abstract



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1. Introduction

Beetroot (Beta vulgaris L.) is in growing demand for wholesome and practical food choices. In the food industry beetroot powder is used as a natural food colouring agent and a versatile ingredient with various culinary applications. A natural source of vegetable waste contains a number of bioactive compounds, which makes it attractive for producing value- added products. Paper from newspring and magazines, which are part and parcel of our daily lives, has been used on a large scale in food packaging. Synthetic additives like antioxidant and photo-initiator compounds are used widely for the production of printing inks, which pollute the environment (Liu & Mabury, 2021). Besides polluting the environment, certain constituents of synthetic ink can adversely impact human health as most of them are carcinogenic and toxic in nature (Affat, 2021) [1-3]. The three main components of ink are the liquid vehicle which facilitates the flow of the dye to the paper, the colourant responsible for imparting the desired colour to the paper, and the additive responsible for enhancing the writing performance of the ink (Ferretti et al, 2023) [4]. Some pigments from hematein and sepia fractions have been explored earlier for their use as colourants (Centeno et al, 2010; Roldán et al, 2014). Beetroot is used as a source of food colourant because of its content of betalain pigments like betaxanthin, betanin and betanidin. Among these, betanin is abundantly used due to its higher content in beetroot (Singh et al, 2017) [5]. This pigment is found to be more stable under varying pH and temperature conditions (Costa et al, 2017) [6]. Betalain can be obtained from beetroot waste in an eco-friendly manner, countering another environmental menace caused by food waste (Zvitov & Nussinovitch, 2005) [7]. Conventionally, betanin has been used in many industries for various staining applications in inks, wood, wool and thread (Yeniocak et al, 2015) [8]. Natural and synthetic polymers like sodium alginate, chitosan, silk fibroin, collagen, polylactic acid and polyethylene glycol may be used for the production of bioinks used in the 3D printing of organs and tissues (Budharaju et al, 2024) [9]. Carboxymethyl cellulose (CMC) is a derivative of cellulose and an anionic polyelectrolyte that is commonly employed in various industries as a water-binding, emulsifying, thickening, and stabilizing agent (Barras et al, 2017) [10-13]. PVA is another such polymer binder by virtue of its higher tensile strength, flexibility and mechanical properties (Ahammed & Ayyappan Susila, 2022) [14]. The addition of CMC increases the viscosity of the ink while PVA efficiently gels with the colourants, implying the roles of both of these polymers in formulating better pigment-based coating colours (Choi et al, 2015) [15-17]. This study focused on obtaining a pigment extract from waste beetroot peel and the use of the extract for formulating bioink. The concentrations of CMC and PVA used for the formulation of bioink were optimised using response surface methodology analysis by recording the drying time of the ink [18-21].

2. Materials and Methodology

Beetroot peel waste was collected from a canteen located within the premises of Kalasalingam Academy of Research and Education (Latitude: 9.5747, Longitude: 77.6798). The chemicals used for the preparation of beetroot oeel extract and formulation of bioink were ethanol, carboxymethyl cellulose (CMC), polyvinyl alcohol (PVA) and water. All reagents used in the study were of high purity (>90%) [22].

2.1. Preparation of Aqueous Beetroot Peel Extract

The beetroot peel was cleaned, dried in the shade, and ground to fine particles which were used for preparing the extract (Borjan et al, 2022). 5 g of finely ground beetroot peel particles were added to 50 ml of 80% (v/v) ethanol and the suspension incubated on a rotary shaker for 4 hr [23]. After the incubation, the suspension was centrifuged at 5000 rpm and the supernatant filtered to obtain the extract (Alameri, 2021; Flores-Mancha et al, 2020).

2.2. Preparation of CMC and PVA Solutions

Carboxymethyl cellulose (CMC) and polyvinyl alcohol (PVA) solutions were prepared at concentrations of 15% (w/v) and 2.5% (w/v), respectively, by dissolving them in water [24-27]. The solutions were stirred for 30 min using a magnetic stirrer for complete dissolution of solute

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particles (Diaz-Gomez et al, 2022; Monne et al, 2021) [28-31].

2.3. Response Surface Methodology for Ink Preparation

To investigate the relationship between drying time and the concentrations of components of the bioink, response surface methodology (RSM) with central composite design (CCD) was used (Wu, J., et al, 2023 [32]; Horlor, B., et al, 2020) [33]. For three parameters (Concentration of CMC, PVA and beetroot extract), the Design Expert software suggested 20 trial runs with six centre points. The low and high values for the parameters set in the trial run are shown in Table 1.

Table 1 High and Low values of the variables in the study

Components	High Value	Low Value
Extract Concentration (ml)	70	60
CMC Concentration (ml)	10	30
PVA Concentration	10	30

All the trial were performed individually and drying time in sec was recorded as response and the results were subjected to ANOVA analysis.

3. Results and Discussion

3.1. Results

The optimum drying time of the bioink produced from beetroot peel waste was analysed using response surface methodology. On performing ANOVA, an F-value of 101.25 and a p-value of <0.0012 resulted, indicating the significance of the model. These mathematical models were found to be statistically significant and considered the best for the results (Talaei et al, 2023) [34]. The coefficients of multiple determinations (R^2) and the adjusted statistic coefficient (R^2_{adj}) were found to be in agreement with both the responses (Nobandegani et al, 2016). The R^2 value of the model shows that 87% of the variability in responses was considered, thereby validating the proposed model for use in the synthesis of bioink. The drying time of the bioink produced may be evaluated from the following equation as

$$\frac{1}{\text{Drying time}} = 0.013486 - (0.000077 * \text{Extract concentration}) + (0.000035 * \text{CMC concentration}) + (0.000035 * \text{PVA concentration})$$

The optimal parameters from obtained from the response surface methodology is shown in Figure 1.

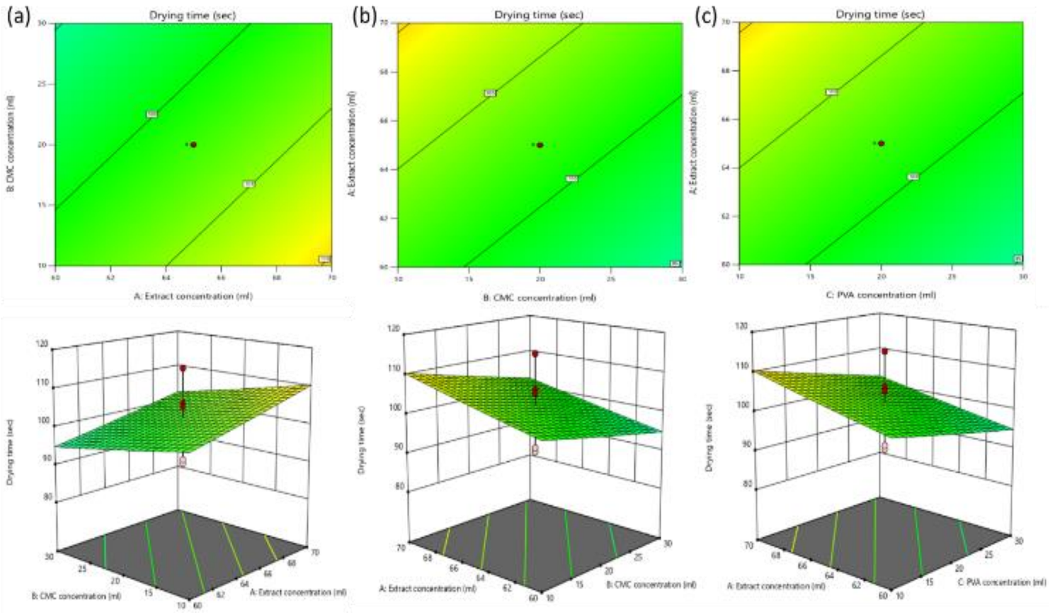

Figure 1 Contour plots and 3D surface plot of optimisation study (a) Extract concentration (ml) Vs Drying time of extract (sec), (b) CMC concentration (ml) Vs Drying time of CMC (sec), (c) PVA concentration (ml) Vs Drying time of PVA (sec)

3.2. Discussion

An optimum drying time of 91.713 sec was obtained upon adopting a combination of 60 ml of extract, 30 ml of CMC solution and 30 ml of PVA solution. Optimal concentration of the 60-ml extract showed the appropriate colour of the ink formulated (Taha et al, 2011). The increased concentration of carboxymethyl cellulose reflects its role as a multifunctional additive that possess both binding and dispersing properties for the formulation of paints (Lamnini et al, 2022). CMC has been employed recently in the preparation of the structural components of bioinks due to its exquisite matrix-forming ability and feasibility of cross-linking with other polymeric materials (Mallakpour et al, 2021). Similar to CMC, a higher concentration of PVA was found to be effective in the preparation of bioinks due to the latter's viable properties. PVA possesses stronger hydrophilic properties due to the presence of multiple hydroxyl groups, which enhance its water-binding capacity and which, in turn, aids in its physical stability (Joorabloo et al, 2019). PVA is used as a stronger binding agent in coatings for boards and papers, mostly in inkjet printing, through the formation of multiple layers over a solid surface (Svanholm & Ström, 2004; Salaoru et al, 2017). It has been found that polymeric blends prepared from CMC and PVA possess synergistic effects with improved properties. The water absorption property of CMC/ PVA composites was enhanced due to structural deformations exposing multiple hydrophilic groups (Arefian et al, 2020). The denser and tougher layers formed with the CMC/ PVA blend have a 77.8% critical cracking thickness, rendering it suitable for the formulation of coating suspensions (Oh et al, 2022). The betacyanin and anthocyanin present in beetroot extract turn red in acidic and neutral pH conditions (Liu et al, 2023). Therefore, this can be exploited for the formulation of biodegradable paints from beetroot peels.

Conclusion

Beetroot peel from food waste was used for the formulation of a bioink. The bioink formulated had biodegradable components, making it safer than the commercially available printing ink containing synthetic carcinogens. Thus, the bioink produced from beetroot waste peel can be explored further for real-world applications.

Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Ms. Shalini Mohan, Senior Research Fellow, acknowledges the Department of Biotechnology (DBT), Government of India, for the financial grant offered with the fellowship ID DBT/2022-23/KARE/2059. Dr. Lakshmanan Muthulakshmi acknowledges the Department of Scientific and Industrial Research for the financial grant (DSIR/TDUPW-1101/3/2021-IRD (SC)/-DSIR, and Kalasalingam Academy of Research and Education for the seed money grant for the year 2021-2022.

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