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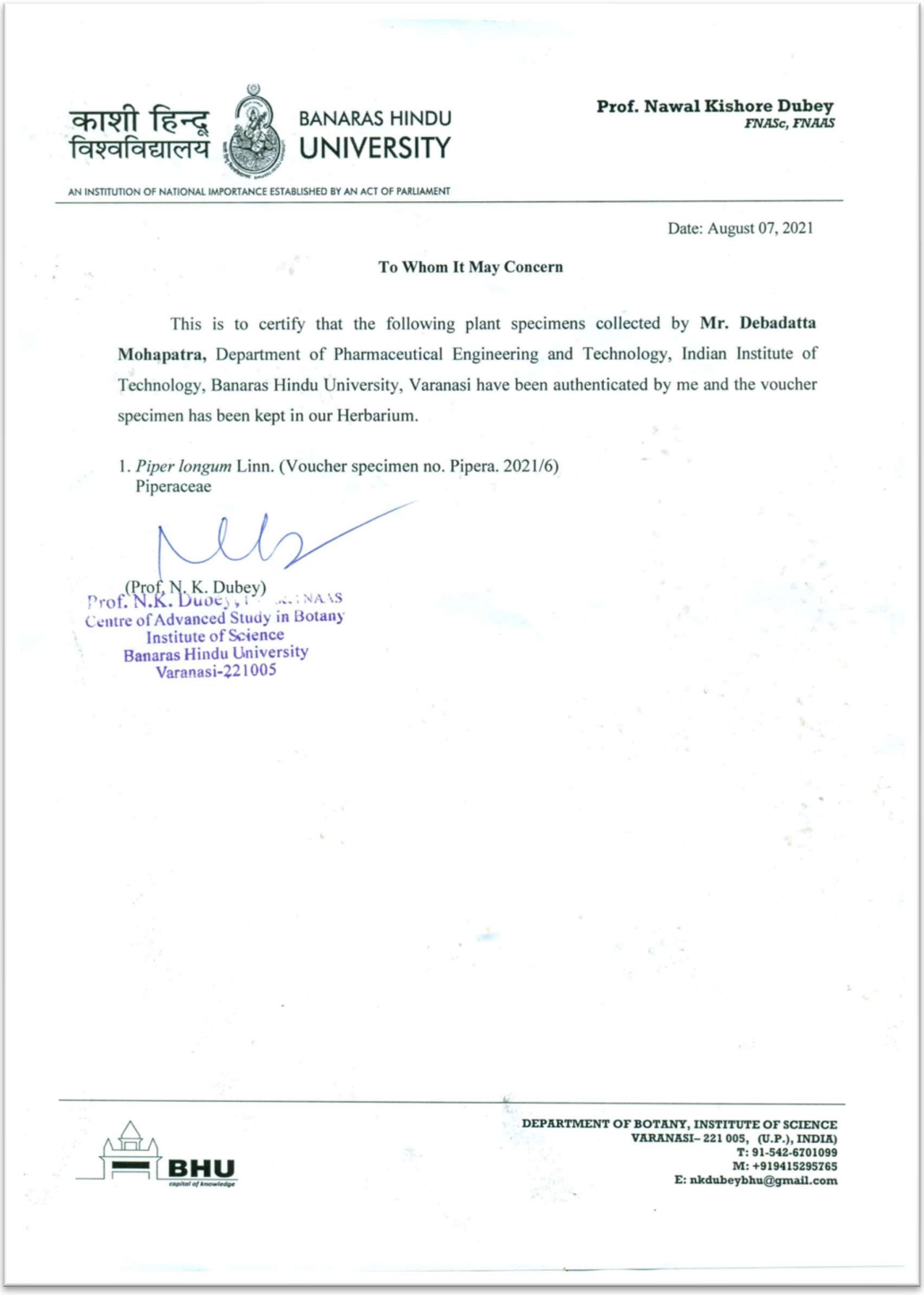
10 Appendices

10.1 Appendix A: Taxonomical authentication of *Piper longum* fruits



काशी हिन्दू विश्वविद्यालय BANARAS HINDU UNIVERSITY

AN INSTITUTION OF NATIONAL IMPORTANCE ESTABLISHED BY AN ACT OF PARLIAMENT

Prof. Nawal Kishore Dubey
FNASc, FNAAS

Date: August 07, 2021

To Whom It May Concern

This is to certify that the following plant specimens collected by **Mr. Debadatta Mohapatra,** Department of Pharmaceutical Engineering and Technology, Indian Institute of Technology, Banaras Hindu University, Varanasi have been authenticated by me and the voucher specimen has been kept in our Herbarium.

1. *Piper longum* Linn. (Voucher specimen no. Pipera. 2021/6)
   Piperaceae

(Prof. N. K. Dubey)
Prof. N.K. Dubey, FNASc, FNAAS
Centre of Advanced Study in Botany
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10.2 Appendix B: Institutional Animal Ethic Committee (IAEC) Certificates

भारतीय प्रौद्योगिकी संस्थान
काशी हिन्दू विश्वविद्यालय
IIT INDIAN INSTITUTE OF TECHNOLOGY BANARAS HINDU UNIVERSITY

Department of Pharmaceutical Engineering & Technology

Regd. No. 2123/GO/Re/S/21/CPCSEA Date: 03 May, 2022

IAEC Approval Number: **IIT(BHU)/IAEC/2022/001**

CERTIFICATE

This is to certify that the project proposal entitled **"Novel Phytoformulations for Melanoma Cancer Therapy"** submitted by **Mr. Debadatta Mohapatra** under supervision of **Dr. Alakh Niranjan Sahu** has been approved/recommended by the IAEC of *Indian Institute of Technology, Banaras Hindu University, Varanasi* in its meeting dated **03/05/2022** and has been sanctioned **70 Male/Female C57BL6 Mice (20-25 gm)** under this proposal for a duration of **Twelve (12) months.**

Prof. Sushant Kumar Shrivastava	Dr. Vinod Tiwari	Dr. Shesh Narayan Mishra
Name & Signature	**Name & Signature**	**Name & Signature**
Chairman	**Member Secretary**	**Main Nominee of CPCSEA**

Note: The CPCSEA Guideline should be followed strictly while handling the animals.

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IIT INDIAN INSTITUTE OF TECHNOLOGY
BANARAS HINDU UNIVERSITY

Department of Pharmaceutical Engineering & Technology

Regd. No. 2123/GO/Re/S/21/CPCSEA

Date: 09 Feb,2023

IAEC Approval Number: **IIT(BHU)/IAEC/2023/056**

CERTIFICATE

This is to certify that the project proposal entitled **"Novel Phytoformulations for Melanoma Cancer Therapy"** submitted by **Mr. Debadatta Mohapatra** under supervision of **Dr. Alakh N. Sahu** has been approved/recommended by the IAEC of Indian Institute of Technology, Banaras Hindu University, Varanasi in its meeting dated **09/02/2023** and has been sanctioned **50 C57BL/6 Mice and 20 SD Rats female** under this proposal for a duration of **Twelve (12) months.**

Prof. Vikash Kumar Dubey	Dr. Vinod Tiwari	Dr. Shesh Narayan Mishra
Name & Signature	**Name & Signature**	**Name & Signature**
Chairman	**Member Secretary**	**Main Nominee of CPCSEA**

Note: The CPCSEA Guideline should be followed strictly while handling the animals

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Department of Pharmaceutical Engineering & Technology

Regd. No. 2123/GO/Re/S/21/CPCSEA

Date: 25 August,2023

IAEC Approval Number: **IIT(BHU)/IAEC/2023/II/052**

CERTIFICATE

This is to certify that the project proposal entitled "***Development and Characterization of Novel Phytoformulations for Melanoma Therapy***" submitted by **Debadatta Mohapatra** under supervision of **Dr. A N Sahu** has been approved/recommended by the IAEC of Indian Institute of Technology, Banaras Hindu University, Varanasi in its meeting dated **25 August, 2023** and has been sanctioned **50 (Female C57BL/6 Mice)** under this proposal for a duration of **12 (Twelve) months.**

Prof. Vikash Kumar Dubey	Dr. Vinod Tiwari	Dr. Shesh Narayan Mishra
Name & Signature	**Name & Signature**	**Name & Signature**
Chairman	**Member Secretary**	**Main Nominee of CPCSEA**

Note: The CPCSEA Guideline should be followed strictly while handling the animals.

10.3 Appendix C: Acute oral toxicity report of standardized extract

AOT425statpgm (Version: 1.0) Test Results and Recommendations
Acute Oral Toxicity (OECD Test Guideline 425) Statistical Program

Date/Time: 17 July 2022, 12:25:51
Data file name: Toxicity_002.dat
Last modified: 17-07-2022 12:21:38

Test/Substance: Acute oral toxicity of standardized PLFEE
Test type: Main Test
Limit dose (mg/kg): 2000
Assumed LD50 (mg/kg): Default
Assumed sigma (mg/kg): 0.5

Recommended dose progression: 2000, 550, 175, 55, 17.5, 5.5, 1.75

DATA:

Test Seq.	Animal ID	Dose (mg/kg)	Short-term Result	Long-term Result
1	PLFEE-1	175	O	O
2	PLFEE-2	550	O	O
3	PLFEE-3	2000	X	X
4	PLFEE-4	550	O	O
5	PLFEE-5	2000	X	X
6	PLFEE-6	550	O	O
7	PLFEE-7	2000	X	X

(X = Died, O = Survived)

Dose Recommendation: The main test is complete.

Stopping criteria met: 5 reversals in 6 tests.

SUMMARY OF LONG-TERM RESULTS:

Dose	O	X	Total
175	1	0	1
550	3	0	3
2000	0	3	3
All Doses	4	3	7

Statistical Estimate based on long term outcomes:

Estimated LD50 = 1098 (Based on an assumed sigma of 0.5).
Approximate 95% confidence interval is 550 to 2000.

10.4 Appendix D: Publications

10.4.1 List of publications from the Thesis work

1. **Mohapatra, Debadatta**, Dulla Naveen Kumar, Singh Shreya, Dhananjay Panigrahi, Ashish Kumar Agrawal, and Alakh N. Sahu. "Quality-by-design-based development of ultradeformable nanovesicular transgelosome of standardized *Piper longum* extract for melanoma." Nanomedicine 0 (2023). https://doi.org/10.2217/nnm-2023-0069
2. **Mohapatra, Debadatta**, Dulla Naveen Kumar, Singh Shreya, Vivek Pandey, Pawan K. Dubey, Ashish Kumar Agarwal, and Alakh N. Sahu. "Quality by design-based development and optimization of fourth-generation ternary solid dispersion of standardized *Piper longum* extract for melanoma therapy." Drug Delivery and Translational Research, (2023). https://doi.org/10.1007/s13346-023-01375-y
3. Sahu, Alakh N., and **Debadatta Mohapatra**. "Nanovesicular transferosomes for the topical delivery of plant bioactives." Nanomedicine 16, no. 28 (2021): 2491-2495. https://doi.org/10.2217/nnm-2021-0316
4. **Mohapatra, Debadatta**, Ashish K. Agrawal, and Alakh N. Sahu. "Exploring the potential of solid dispersion for improving solubility, dissolution & bioavailability of herbal extracts, enriched fractions, and bioactives." Journal of Microencapsulation 38, no. 7-8 (2021): 594-612. https://doi.org/10.1080/02652048.2021.1963342

10.4.2 List of other publications during the research work

10.4.2.1 Research articles

1. Sahu, Alakh N., **Debadatta Mohapatra**, and Pratap Chandra Acharya. "Nanovesicular ultraflexible invasomes and invasomal gel for transdermal delivery of phytopharmaceuticals." Nanomedicine 0 (2024). https://doi.org/10.2217/nnm-2024-0029
2. Naik, Gaurav Gopal, Ravi Pratap, **Debadatta Mohapatra**, Singh Shreya, Deepak K. Sharma, Avanish S. Parmar, Arjun Patra, and Alakh N. Sahu. "From phytomedicine to photomedicine: quercetin-derived carbon nanodots—synthesis, characterization and healthcare applications." Journal of Materials Science 58, no. 34 (2023): 13744-13761. https://doi.org/10.1007/s10853-023-08880-y
3. H. V. Chethan, **Debadatta Mohapatra**, Alakh N Sahu and Siva Hemalatha. "Formulation Development and Evaluation of Hydrogel Containing Silver Nanoparticles with *Withania coagulans* Aqueous Extract." Indian Journal of Pharmaceutical Sciences 85, no. 4 (2023): 987-996, https://doi.org/10.36468/pharmaceutical-sciences.1165
4. Mishra, Krishna N., **Debadatta Mohapatra**, Pratibha Chaubey, Alakh N. Sahu, Shailendra Kumar, and Harish C. Upadhyay. "Bio-fabricatiton of Silver Nanoparticles Using *Alysicarpus vaginalis* Extract: Preparation, Characterization and Comparative in vitro Antibacterial Evaluations." ChemistrySelect 8, no. 24 (2023): e202301113. https://doi.org/10.1002/slct.202301113
5. Shreya, Singh, **Debadatta Mohapatra**, Gaurav Gopal Naik, Yamini Bobde, Balaram Ghosh, and Alakh N. Sahu. "In vitro Antioxidant and Cytotoxic Potential of *Pleurotus* Mushroom and Activity-Based Correlation: a Comparative Study." Journal of Analytical Chemistry 78, no. 4 (2023): 456-463. https://doi.org/10.1134/S1061934823040135

6. **Mohapatra, Debadatta**, Ravi Pratap, Vivek Pandey, Singh Shreya, Gaurav Gopal Naik, Subhash C. Mandal, Sunday O. Otimenyin, Pawan K. Dubey, Avanish S. Parmar, and Alakh N. Sahu. "Bioengineered dual fluorescent carbon nanodots from Indian Long pepper leaves for multifaceted environmental and health utilities." Environmental Science and Pollution Research 30, no. 18 (2023): 52182-52208. https://doi.org/10.1007/s11356-023-25887-9
7. Shreya, Singh, Deepak Kasote, **Debadatta Mohapatra**, Gaurav Gopal Naik, Santosh Kumar Guru, Nese Sreenivasulu, Yashpal Sharma, and Alakh N. Sahu. "Chemometric-Based Analysis of Metabolomics Studies of Bioactive Fractions of *Pleurotus osteratus* and Their Correlation with In Vitro Anti-Cancer Activity." Applied Biochemistry and Biotechnology (2023): 1-15. https://doi.org/10.1007/s12010-023-04325-z
8. Shreya, Singh, Dulla Naveen Kumar, **Debadatta Mohapatra**, Shivani Jaiswal, Gaurav Gopal Naik, Santosh Kumar Guru, Ashish Kumar Agarwal, Senthil Raja Ayyannan, and Alakh N. Sahu. "Tracing the anti-cancer mechanism of *Pleurotus osteratus* by the integrative approach of network pharmacology and experimental studies." Applied Biochemistry and Biotechnology 195, no. 1 (2023): 152-171. https://doi.org/10.1007/s12010-022-04111-3.
9. Dixit, Kohina, **Debadatta Mohapatra**, P. C. Senapati, and A. N. Sahu. "Formulation Development and Evaluation of *Lawsonia inermis* Extract Loaded Hydrogel for Wound Dressing Application." Indian Journal of Pharmaceutical Sciences 84, no. 4 (2022): 848-862.
10. **Mohapatra, Debadatta**, Ravi Pratap, Vivek Pandey, Singh Shreya, Prakash Ch Senapati, Pawan K. Dubey, Avanish S. Parmar, and Alakh N. Sahu. "In vitro cancer cell imaging, free radical scavenging, and Fe3+ sensing activity of green synthesized carbon dots from leaves of *Piper longum*." Journal of Cluster Science 34, no. 3 (2023): 1269-1290. https://doi.org/10.1007/s10876-022-02303-9
11. **Mohapatra, Debadatta**, Ravi Pratap, Vivek Pandey, Pawan K. Dubey, Ashish K. Agrawal, Avanish S. Parmar, and Alakh N. Sahu. "*Tinospora Cordifolia* Leaves Derived Carbon Dots For Cancer Cell Bioimaging, Free Radical Scavenging, And Fe3+ Sensing Applications." Journal of Fluorescence (2022): 1-18. https://doi.org/10.1007/s10895-021-02846-6
12. **Mohapatra, Debadatta**, Md Bayazeed Alam, Vivek Pandey, Ravi Pratap, Pawan K. Dubey, Avanish S. Parmar, and Alakh N. Sahu. "Carbon dots from an immunomodulatory plant for cancer cell imaging, free radical scavenging and metal sensing applications." Nanomedicine 16, no. 23 (2021): 2039-2059. https://doi.org/10.2217/nnm-2021-0190
13. Naik, Gaurav Gopal, Md Bayazeed Alam, Vivek Pandey, **Debadatta Mohapatra**, Pawan K. Dubey, Avanish S. Parmar, and Alakh N. Sahu. "Multi-Functional Carbon Dots from an Ayurvedic Medicinal Plant for Cancer Cell Bioimaging Applications." Journal of Fluorescence (2020): 1-12. https://doi.org/10.1007/s10895-020-02515-0

10.4.2.2 Book

1. Alekh Niranjan Sahu & **Debadatta Mohapatra**, "Herbal Drug Formulation and Standardization" Ist Edition 2021, Ane Books Pvt. Ltd., 4821, Parwana Bhawan, 1st Floor, 24 Ansari Road, Darya Ganj, New Delhi - 110 002. ISBN-10: 9390658365, ISBN-13: 978-9390658367. Published on 21 June 2021.

Research Article

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Nanomedicine

Quality-by-design-based development of ultradeformable nanovesicular transgelosome of standardized *Piper longum* extract for melanoma

Debadatta Mohapatra[1], Dulla Naveen Kumar[2], Singh Shreya[1], Dhananjay Panigrahi[3], Ashish Kumar Agrawal[2] & Alakh N Sahu*,[1]

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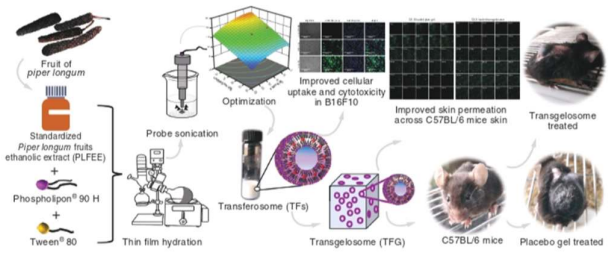
Background: Melanoma is the most aggressive and deadly form of skin cancer. The stratum corneum of the skin is a major obstacle to dermal and transdermal drug delivery. Ultradeformable nanovesicular transferosome has the capacity for deeper skin penetration and its incorporation into hydrogel forms a transgelosome that has better skin permeability and patient compliance. **Method:** Here, the quality-by-design-based development and optimization of nanovesicular transgelosome of standardized *Piper longum* fruit ethanolic extract (PLFEE) for melanoma therapy are reported. **Results:** Compared with standardized PLFEE-loaded plain gel, the transgelosome displayed optimal pharmaceutical properties and improved *ex vivo* skin permeability and *in vivo* tumor regression in B16F10 melanoma-bearing C57BL/6 mice. **Conclusion:** The results reflect the potential of transgelosome for melanoma therapy.

Plain language summary: Melanoma is a deadly form of skin cancer that originates from melanocytes in the skin. Skin is a major barrier to drug delivery. Transferosome is a liquid nanoformulation that has the capacity for deeper skin penetration. The transferosome was prepared from standardized *Piper longum* fruit ethanolic extract (PLFEE) and loaded into gel to form a transgelosome for improved skin application and patient compliance. Compared with extract-loaded plain gel, the transgelosome showed good pharmaceutical properties with better activity in melanoma (B16F10)-bearing female C57BL/6 mice. The therapeutic activity of the standard anticancer drug dacarbazine was improved with the prepared PLFEE transgelosome.

Graphical abstract:



First draft submitted: 11 March 2023; Accepted for publication: 6 July 2023; Published online: 28 July 2023

Keywords: central composite design (CCD) • formulation and development • *in vivo* • melanoma • optimization • *Piper longum* extract • standardization • transferosome • transgelosome

Future Medicine

10.2217/nnm-2023-0069 © 2023 Future Medicine Ltd **Nanomedicine (Lond.)** (Epub ahead of print) ISSN 1743-5889

Drug Delivery and Translational Research
https://doi.org/10.1007/s13346-023-01375-y

ORIGINAL ARTICLE

Quality by design–based development and optimization of fourth-generation ternary solid dispersion of standardized *Piper longum* extract for melanoma therapy

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Accepted: 25 May 2023

Abstract
The study aimed to enhance the solubility, dissolution, and oral bioavailability of standardized *Piper longum* fruits ethanolic extract (PLFEE) via fourth-generation ternary solid dispersion (SD) for melanoma therapy. With the use of solvent evaporation method, the standardized PLFEE was formulated into SD, optimized using Box-Wilson's central composite design (CCD), and evaluated for pharmaceutical performance and in vivo anticancer activity against melanoma (B16F10)–bearing C57BL/6 mice. The optimized SD showed good accelerated stability, high yield, drug content, and content uniformity for bioactive marker piperine (PIP). The X-ray diffraction (XRD), differential scanning calorimetry (DSC), polarized light microscopy (PLM), and selected area electron diffraction (SAED) analysis revealed its amorphous nature. The attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) and high-performance thin layer chromatography (HPTLC) revealed the compatibility of excipients with the PLFEE. The contact angle measurement and in vitro dissolution study revealed excellent wetting of SD and improved dissolution profile as compared to the plain PLFEE. The in vivo oral bioavailability of SD reflected a significant ($p < 0.05$) improvement in bioavailability ($F_{rel} = 188.765\%$) as compared to plain extract. The in vivo tumor regression study revealed the improved therapeutic activity of SD as compared to plain PLFEE. Further, the SD also improved the anticancer activity of dacarbazine (DTIC) as an adjuvant therapy. The overall result revealed the potential of developed SD for melanoma therapy either alone or as an adjuvant therapy with DTIC.

Keywords Solid dispersion · Optimization · *Piper longum* · Dissolution · Bioavailability · Melanoma

Introduction

Melanoma, a most aggressive and deadly form of skin cancer, arises from the malignant transformation of melanocytes. As per National Cancer Institute (NCI) epidemiology survey, melanoma is the 5th most common cancer in USA with an estimated 97,610 new cases and 7,990 deaths in 2023 [1]. Data from the Global Cancer Observatory (GCO) shows that the annual incidence of melanoma cancer in both sexes in 2020 was 3,24,635 cases worldwide, with the highest number recorded in Europe (150,627), followed by Northern America (105,172), Asia (23,753), Oceania (19,239), Latin America and the Caribbean (18,881), and Africa (6963) [2]. Fair-skinned Caucasian populations are more prone to melanoma; however, its occurrence in pigmented populations in Asia and Africa has also been noticed on the nail beds, mucous membranes, and soles of the feet at a low incidence rate [3, 4]. The most prevalent type of melanoma is cutaneous melanoma which appears on the cutaneous surface [5, 6]. Although melanoma is regarded as multifactorial, the major risk factor is excessive exposure to ultraviolet (UV) radiation, which causes genetic mutations, and DNA damage, and mediates inflammatory responses [3, 4, 6]. In

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Published online: 09 June 2023

Editorial

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Nanomedicine

Nanovesicular transferosomes for the topical delivery of plant bioactives

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"Lipid-based vesicular nano-formulations, especially TFs, have gained attention among other vesicular carriers due to their self-optimizing deformability properties, allowing them to penetrate to the deeper skin layers without the rupture of vesicles or loss of vesicular content."

First draft submitted: 24 August 2021; Accepted for publication: 18 October 2021; Published online: 8 November 2021

Keywords: formulation • nanovesicular system • transferosomes • natural products • plant bioactives • plant extract • topical delivery • transdermal drug delivery

Natural products play a substantial role in the holistic healthcare of human beings from the ancient era to date. Around 506 newly approved pharmaceuticals from 1981 to 2019 are either natural products or natural product derivatives [1]. These are reliable, safe, efficacious and readily acceptable sources of medicaments [2]. Most patients rely on plant-based medicaments for their primary healthcare needs due to them having fewer side effects [3]. Herbal drugs may either be whole plant parts, multicomponent-based extracts, enriched fractions or isolated bioactives [2]. Topical drug administration offers noninvasive site-specific drug delivery, greater patient convenience, extended duration of the activity, reduced side effects and avoidance of gastric degradation, gastric irritation and first-pass metabolism [4,5]. However, the *stratum corneum* is a major obstacle to dermal and transdermal drug delivery because it limits drug transport across the skin and makes the topical route inefficient for medical use. Ionized drug candidates and molecules with molecular weights greater than 500 Da generally do not cross through the skin [4,6,7]. Some plant bioactives possess poor lipid solubility and low transmembrane permeability [8]. Despite the excellent *in vitro* bioactivity of phytoconstituents, their low lipid solubility, larger molecular size and complex structure compared with synthetic compounds restrict the permeation across the lipid-rich biological membrane during topical administration [9]. Furthermore, some plant bioactives, such as flavonoids, possess poor aqueous solubility, inadequate dissolution, poor oral absorption, limited oral bioavailability and gastric degradability properties [7]. Such challenges may be addressed through novel topical formulations such as micro/nanoemulsions, nanofibers, nanostructured lipid carriers, solid lipid nanoparticles, phytosomes, liposomes, ethosomes, cubosomes and noisomes. However, transferosomes (TFs) have been noted as the most promising novel topical formulations for improving transdermal permeability [10].

Transferosomes: composition, penetration mechanism, methods of preparation & advantages

TFs are a patented product of the company IDEA AG (Munich, Germany). The name is derived from the Latin word "*transferre*", which means "*to carry across*," and the Greek word "*soma*," which means a "*body*" or "*cell-like*" [6,10,11]. Transferosomes are novel ultradeformable vesicular formulations primarily fabricated from three basic components, namely phospholipids, surfactant/edge activators and water [6,11]. Ethanol may sometimes be incorporated into the transferosomal formulation [10]. Phospholipids (usually above 70%), such as phosphatidylcholine (egg phosphatidylcholine and soybean phosphatidylcholine), dipalmitylphosphatidylcholine, dimyristoyl phosphatidylcholine, distearylphosphatidylcholine and dipalmitoyl phosphatidylglycerol are commonly employed for the formulation of TFs [6,10]. Various edge activators (ranging from 10 to 25%) such as a surfactant (e.g., Tween® 20, Tween® 60, Tween® 80, Span® 60, Span® 65 and Span® 80) or bile salt (e.g., sodium cholate and sodium deoxycholate) are generally incorporated into the TFs. They make the membrane flexible and ultradeformable and

Future Medicine

10.2217/nnm-2021-0316 © 2021 Future Medicine Ltd *Nanomedicine (Lond.)* (Epub ahead of print) ISSN 1743-5889

Journal of Microencapsulation
Micro and Nano Carriers

Taylor & Francis

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/imnc20

Exploring the potential of solid dispersion for improving solubility, dissolution & bioavailability of herbal extracts, enriched fractions, and bioactives

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To cite this article: Debadatta Mohapatra, Ashish K. Agrawal & Alakh N. Sahu (2021) Exploring the potential of solid dispersion for improving solubility, dissolution & bioavailability of herbal extracts, enriched fractions, and bioactives, Journal of Microencapsulation, 38:7-8, 594-612, DOI: 10.1080/02652048.2021.1963342

To link to this article: https://doi.org/10.1080/02652048.2021.1963342

10.5 Appendix E: Skills learned during Ph.D. tenure

10.5.1.1 Instrumental skills

Handling, method development, validation, data analysis, and interpretation of

- *In-vivo* bioimager (PhotonIMAGER Optima, Biospacelab, France)
- HPTLC (CAMAG, Switzerland)
- HPLC (Agilent 1260 Infinity II, Agilent, USA; 1525 Waters® Corporation, Milford, MA, USA)
- Fluorescence spectrophotometer (Fluorolog-Horiba, Jobin Yvon, France)
- Rheometer (DV-II+ digital viscometer, Brookfield Engineering, USA; MCR 72, Anton Paar GmbH, Austria)
- FTIR (Bruker Alpha II, Germany; Shimadzu. Japan)
- Particle size analyzer (Zetasizer Pro, Malvern Panalytical Ltd., UK)
- Dissolution apparatus IP/BP/USP:(Electrolab, India)
- Contact angle/ Drop shape analyzer (DSA25S, KRÜSS GmbH, Hamburg, Germany)
- Biochemistry analyzer (CHEM-5 Plus v2, Erba, Mannheim, Germany)
- Polarized Light Microscopy (Radical RXLr-5, New Delhi, India)
- Inverted microscopy (Victory Plus, Dewinter, New Delhi, India)
- Optical microscopy (Magnus MLX Plus, Olympus Opto Systems India Pvt. Ltd., Uttar Pradesh, India)
- Multimode reader (SpectraMax M5, Molecular Devices, USA; Bio-Rad Laboratories, Munchen, Germany)
- DSC equipment (DSC 60 Plus, Shimadzu, Japan)
- TGA apparatus (Shimadzu TGA-50 analyzer, Shimadzu, Japan)

10.5.1.2 Software skills

Statistical and graphical programming software

- Origin (Version 9.8.0.200, OriginLab Corporation, MA, USA)
- GraphPad Prism (Version 5.01, GraphPad Software, Inc., San Diego, USA)
- Image J (NIH, Bethesda, Maryland)
- PK Solver (Microsoft Corporation, USA)

Optimization software

- Design Expert (Version 12, Stat-Ease, MN, USA)

Chemical graphics software

- ChemDraw Ultra (Version 12.0.2, USA)

Image editing and processing software

- Adobe Photoshop (Version 21.2.2 20200807, USA)

In-silico docking and visualization software

- Auto Dock Vina (Version 1.2.0, Scripps Research Institute, USA)
- BIOVIA Discovery Studio (Version 4.5, Dassault Systemes BIOVIA, USA)

Referencing software

- Endnote (Version: Bld12062, ClarivateTM, USA)

Instrumental software

- FluorEssence TM (Version 3.5.1.2, Horiba, HORIBA, France) for Fluorescence
- WinCATS software (Version 1.4.7.2018, CAMAG, Switzerland) for HPTLC

- Open LAB CDS EZChrom Workstation VL software (Agilent, USA) for HPLC
- Zen (Blue edition) software (Version 3.6, Carl Zeiss Microscopy, GMBH, Germany) for CLSM
- Opus (Version 7.0, Bruker, Ettlingen, Germany) for ATR-FTIR
- ProCam software (Radical Version 3.7, India) for Polarized light microscopy
- RheoCompassTM software (Version 1.3, Anton Paar GmbH, Austria) for Rheometer
- ZS Xplorer software (Version 2.3.1, Malvern Panalytical Ltd., UK) for Particle size analyzer
- Magvision software (Version x36, 3.7.6820, Olympus Opto Systems India Pvt. Lt d., India) for Optical Microscopy
- Softmax Pro software (Version 3.12, Molecular Devices, USA) for Multimode reader

10.5.1.3 Academic skills

- Conceptualizing, designing, and writing founded project proposal
- Mentoring UG and PG students
- Teaching assistant to various subjects in UG and PG courses
- Documentation (Inventory management, stock, purchase, bills, etc.)

10.5.1.4 Independent experimental designing, feasibility analysis, and execution

- Conduction of pharmaceutical, analytical, and biological experiments
- Data analysis, validation, and interpretation
- Independent planning, manuscript writing, and communication

10.5.1.5 Presentations

- Poster presentation on "Development and characterization of *Piper longum* extract-based solid dispersion for melanoma therapy" in the 3rd International Conference On "Innovations in Chemical, Biological and Pharmaceutical Sciences (ICBPS-2023), held on 23-25th November 2023 organized by GLA University, Mathura, Uttar Pradesh, India.
- Poster presentation on "Development and characterization of ultra-flexible nanovesicular transgelosome of *Piper longum* for melanoma therapy" in International Conference on "Drug Development and Dug Delivery (CD4)" held on 21-22nd November 2023 organised by University of Lucknow, Lucknow, Uttar Pradesh, India.
- Hydrothermal-engineered biomass-derived carbon nanodots for antioxidant and metal-sensing activity International Conference on Nanotechnology for Better Living (ICNBL-2023), NIT Srinagar, Jammu and Kashmir, India
- Exhibition and scientific poster presentation on the theme: Rural Health Care (Rational drug use) on the occasion of "Republic Day 2023 & Establishment Day of the University (Basant Panchami) procession" Organized by: IIT (BHU), Varanasi, Uttar Pradesh, India.

- Cinnamon oil & clove oil based microemulsion & microemulsion based gel of disulfiram for treatment of melanoma presented on "International Conference on Advanced Material for Better Tomorrow (AMBT)-2021" during July 13-17, 2021, organized by by IIT (BHU) in association with Society for Interdisciplinary Research in Materials & Biology (SIRMB).
- Poster presentation on "Antibacterial evaluation and in vitro Antioxidant activity of *Neptunia oleracea*", International Conference on Bioengineering & Regenerative Medicine (ICBR 2020) School of Biochemical Engineering, IIT(BHU) 2020) School of Biochemical Engineering, IIT(BHU) Date 27th February 2020 29th February 2020.

10.5.1.6 Conferences, seminars, and workshops

- One day hands on training on "Particle Size, Shape Analysis assisted by AI/ML in Pharmaceutical Formulation and Development" 29th September 2023 at Department of Pharmaceutical Engineering and Technology, IIT (BHU), Varanasi, Uttar Pradesh, India.
- Rastrita Bigyan Sangosthi, "Aushadh Vigyaan kee Adyatan Praudyogikee: Vartamaan aur Bhavish", 27th May 2023, Department of Pharmaceutical Engineering and Technology, IIT (BHU), Varanasi, Uttar Pradesh, India.
- Webinar on Advanced Research on Phytochemistry and Its Impact, organized by Gitanjali College of Pharmacy on 29th June 2022.
- International Conference on Advanced Material for Better Tomorrow (AMBT)-2021, during July 13-17, 2021, organized by IIT (BHU) in association with the Society for Interdisciplinary Research in Materials & Biology (SIRMB).
- SPARC sponsored International Workshop on Neurobiology of Pain & Itch being organized by IIT (BHU), Varanasi, from 29th June 2021 to 03rd July 2021.
- Workshop on "Basics of Flow Cytometry and its Applications in Biomedical Sciences" at Department of Biotechnology, Babasaheb Bhimrao Ambedkar University (BBAU), Lucknow.
- International Conference on Bioengineering & Regenerative Medicine (ICBR 2020) School of Biochemical Engineering, IIT(BHU) 2020) School of Biochemical Engineering, IIT(BHU) Date 27th February 2020 to 29th February 2020