

Methylene Blue USP (Softgel Preparation)

A single-component SEDDS softgel preparation of Methylene Blue USP (methylthioninium chloride), the phenothiazine-class redox-active small molecule originally synthesised by Heinrich Caro in 1876 — characterised in cellular preparations for effects on mitochondrial electron transport, NO synthase and soluble guanylate cyclase inhibition, MAO-A inhibition, and tau aggregation interference.

CATALOG REFERENCE

BM-SOF-005

FORM FACTOR

Softgel · 12.5 mg per capsule

PACK SIZE

60 capsules · 150 mL bottle

DATE OF ISSUE

May 2026

This research preparation is a single-component softgel formulation of **Methylene Blue USP** (methylthioninium chloride), a phenothiazine-class redox-active dye historically used in chemistry, biology, and medicine since its synthesis by Heinrich Caro in 1876. The molecule was the first fully synthetic compound ever used in medicine and has subsequently been characterised in cellular and biochemical preparations for effects on mitochondrial electron transport (acting as an alternative electron carrier), inhibition of nitric oxide synthase and soluble guanylate cyclase, monoamine oxidase A inhibition, and tau protein aggregation interference. The preparation is supplied at 12.5 mg per softgel capsule, 60 capsules per 150 mL bottle in a cobalt-blue shell that reflects the characteristic colour of the active. **This monograph summarises published cellular pharmacology and preclinical findings for laboratory research reference only.**

01 Active Compound Profile

COMMON DESIGNATION	Methylene Blue · Methylthioninium chloride · MB
COMPOUND CLASS	Phenothiazine-class redox-active small molecule
CAS REGISTRY	61-73-4
MOLECULAR FORMULA	$C_{16}H_{18}ClN_3S$
AVERAGE MOLECULAR MASS	319.85 g · mol ⁻¹
PHARMACOLOGICAL GRADE	USP-grade (United States Pharmacopeia specification) – distinct from industrial-grade methylene blue, with reduced heavy-metal and impurity content
PRIMARY MOLECULAR ACTIONS	Alternative electron carrier at the mitochondrial electron transport chain · NO synthase inhibition · soluble guanylate cyclase inhibition · monoamine oxidase A inhibition · tau aggregation interference

02 Softgel Formulation Specifications

ACTIVE PER SOFTGEL	12.5 mg Methylene Blue USP
PACK SIZE	60 softgels per 150 mL bottle
SHELL	Cobalt-blue softgel (reflects characteristic colour of the active)
ENTERIC COATING	None (immediate-release configuration)
VEHICLE	SEDDS lipid-based fill
ANALYTICAL SPECIFICATION	Methylene Blue USP-grade per United States Pharmacopeia specification

03 Origin and Cellular Pharmacology

METHYLENE BLUE WAS SYNTHESISED BY HEINRICH CARO AT BASF IN 1876 AND WAS THE FIRST FULLY SYNTHETIC compound to enter medical use. The molecule has subsequently been characterised in extensive cellular and biochemical literature. The phenothiazine ring system supports facile redox cycling between the oxidised (blue) and reduced (leuco, colourless) forms, providing the molecular basis for methylene blue's action as an alternative electron

carrier in mitochondrial preparations — bypassing dysfunctional electron transport chain complexes by accepting electrons from NADH and donating them to cytochrome c. Additional characterised actions include inhibition of neuronal NO synthase (with downstream effects on cGMP signalling), inhibition of soluble guanylate cyclase, reversible inhibition of monoamine oxidase A, and interference with tau protein aggregation in cell-free and cellular preparations.

04 Preclinical Findings

SYSTEM	PREPARATION	REPORTED OBSERVATION	REF.
Mitochondrial respiration	Isolated mitochondria preparations	Alternative electron carrier between NADH and cytochrome c; supports respiration in complex I/III-inhibited preparations	1
NOS / sGC inhibition	Vascular smooth muscle preparations	Inhibition of NO-cGMP pathway signalling	2
Tau aggregation	Cell-free tau aggregation assays	Interference with tau filament formation	3
MAO-A inhibition	Cell-free enzyme preparations	Reversible inhibition of monoamine oxidase A	4

F Softgel Formulation Considerations

SEDDS-CLASS SOFTGEL CHEMISTRY

The softgel form factor employs a self-emulsifying drug delivery system (SEDDS) lipid-based vehicle inside a gelatin or modified-gelatin shell. SEDDS formulations consist of isotropic mixtures of oils, surfactants, co-surfactants, and the active compound, which spontaneously form fine oil-in-water emulsions upon contact with aqueous gastrointestinal fluids. This formulation strategy is particularly useful for poorly water-soluble actives, supporting dissolution and absorption from the gastrointestinal lumen. Key softgel-formulation considerations are (a) **shell composition** — gelatin shells are sensitive to moisture and temperature; modified-gelatin and plant-based shell alternatives are sometimes used; (b) **enteric coating** — pH-dependent polymer coatings (e.g., methacrylic acid copolymers) delay capsule disintegration until passage through the acidic stomach, releasing the contents in the more neutral environment of the small intestine; (c) **shell colour** — opacifiers and colourants protect light-sensitive actives and provide product identification; (d) **fill volume** — typical softgel fill volumes range from 0.3 to 1.5 mL per capsule, with corresponding shell sizes selected for the formulation.

05 Laboratory Handling and Storage

SEALED SOFTGELS HELD AT CONTROLLED ROOM TEMPERATURE (15–25 °C), PROTECTED FROM MOISTURE AND DIRECT light. Methylene blue stains skin, fabric, and laboratory surfaces; standard chemistry-handling practices apply. Working quantities are determined by the investigator's experimental design.

06 References

- 1 Atamna H, Nguyen A, Schultz C, et al. Methylene blue delays cellular senescence and enhances key mitochondrial biochemical pathways. *FASEB J*. 2008;22(3):703–712. PMID: 17928358
- 2 Mayer B, Brunner F, Schmidt K. Inhibition of nitric oxide synthesis by methylene blue. *Biochem Pharmacol*. 1993;45(2):367–374. PMID: 7679577
- 3 Wischik CM, Edwards PC, Lai RY, Roth M, Harrington CR. Selective inhibition of Alzheimer disease-like tau aggregation by phenothiazines. *Proc Natl Acad Sci USA*. 1996;93(20):11213–11218. PMID: 8855335
- 4 Ramsay RR, Dunford C, Gillman PK. Methylene blue and serotonin toxicity: inhibition of monoamine oxidase A (MAO A) confirms a theoretical prediction. *Br J Pharmacol*. 2007;152(6):946–951. PMID: 17721552

RESEARCH USE ONLY · NOT FOR HUMAN OR VETERINARY CONSUMPTION

BIOMOD Labs is a research chemical supplier. BIOMOD Labs is not a compounding pharmacy as defined under section 503A of the Federal Food, Drug, and Cosmetic Act, nor an outsourcing facility as defined under section 503B. The compounds described in this document have not been evaluated by the U.S. Food and Drug Administration. The statements in this monograph are not intended to diagnose, treat, cure, or prevent any disease. The material is supplied for laboratory and in-vitro experimental use only. Customers are responsible for compliance with all applicable local, state, and federal regulations governing the handling of research chemicals.