substrate. The products of this decomposition also inhibit the luminescent reaction. Additionally, some coelenterazines have low solubility in different assay buffers or directly into test samples or may exhibit inconsistent reconstitution in different assay buffers. While coelenterazines can be dissolved in an organic solvent prior to dilution into an appropriate buffer solution, the organic solutions of coelenterazine compounds may suffer from instability in storage (both thermal instability and photo-instability). However, while solid coelenterazines and coelenterazine analogs and derivatives (e.g., furimazine) are considerably more stable than organic solutions thereof, they exhibit extremely poor reconstitution speed and efficiency, dissolve inconsistently, and are difficult to employ directly in assays and other methods, especially when non-organic solvents are required. These drawbacks have greatly limited the number and types of applications for which coelenterazine, and its analogs and derivatives, have been developed.

Accordingly, there is a need for new compositions and/or methods for stabilizing, improving the solubility of, and/or increasing the reconstitution efficiencies of luminogenic substrates. In particular, having substrates with improved physical characteristics and/or solubility is beneficial for long-term storage (e.g., ≥12 months at room temperature), assay format(s) compatibility, robustness, and user-friendliness.

Provided herein are compositions and methods for stabilizing and improving the solubility and/or the reconstitution efficiency of a luminogenic substrate such as coelenterazine or an analog or derivative thereof. Characterization of the substrate's chemical integrity and/or reconstitution efficiency within different solid compositions, formulations, and formats was performed using HPLC, absorbance, and mass spectroscopy. Additional functional characterization of the substrate under assay relevant conditions was performed by monitoring bioluminescence via relative light units (RLU) in the presence of the NanoLuc® enzyme.

Provided herein are compositions comprising a compound selected from coelenterazine and an analog or derivative thereof, and a polymer. In some examples, the compound is selected from coelenterazine, coelenterazine-h, coelenterazine-h-h, furimazine, JRW-0238, JRW-1743, and JRW-1744. In some examples, the compound is furimazine. In some examples, the compound is JRW-0238. In some examples, the compound is JRW-1743. In some examples, the compound is JRW-1744. In one aspect, the present disclosure refers to furmimazine and pullulan, wherein the composition is in the form of a lyophilized powder or cake. In one example, there is described a composition comprising a compound selected from coelenterazine, coelenterazine-h, coelenterazine-h-h, furimazine, JRW-0238, JRW-1743, and JRW-1744; and a naturally-occurring biopolymer; wherein the composition is in the form of a lyophilized powder or cake.

CLAIMS

1. A composition comprising:
furimazine; and
pullulan;
wherein the composition is in the form of a lyophilized powder or cake.

2. The composition of claim 1, wherein the composition further comprises a buffer, a surfactant, a reducing agent, a salt, a radical scavenger, a chelating agent, a protein, or any combination thereof.

3. The composition of claim 2, wherein the composition further comprises a buffer selected from a phosphate buffer, tricine, and 2-(*N*-morpholino)ethanesulfonic acid.

4. The composition of claim 2 or claim 3, wherein the composition further comprises a surfactant selected from polysorbate 20, polysorbate 40, and polysorbate 80.

5. The composition of any one of claims 2-4, wherein the composition comprises a reducing agent selected from thiourea and 6-aza-2-thiothymine.

6. The composition of any one of claims 2-5, wherein the composition further comprises a salt selected from sodium chloride and sodium phosphate.

7. The composition of any one of claims 2-6, wherein the composition further comprises a radical scavenger agent selected from ascorbic acid and sodium ascorbate.

8. The composition of any one of claims 2-7, wherein the composition further comprises a chelating agent, and the chelating agent selected from citric acid and trans-1,2-diaminocyclohexane-tetraacetic acid.

9. The composition of any one of claims 2-8, wherein the composition further comprises a protein selected from bovine serum albumin, gelatin, and a polypeptide fraction of highly purified dermal collagen of porcine origin.

10. A method of stabilizing furimazine, comprising contacting the furimazine with an effective amount of pullulan to form a composition, wherein the furimazine is stabilized against thermal decomposition, chemical decomposition, light-induced decomposition, or any combination thereof.

11. A method of improving the solubility of furimazine, comprising contacting the furimazine with an effective amount of pullulan to form a composition, wherein the solubility of the furimazine is improved in an aqueous solution compared to furimazine that has not been contacted with the pullulan.

12. A method of improving the reconstitution rate of furimazine, comprising contacting the furimazine with an effective amount of pullulan to form a composition,

wherein the reconstitution rate for the furimazine is improved compared to furimazine that has not been contacted with the pullulan.

13. A kit comprising the composition of any one of claims 1-9.