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 provides 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.

In some examples, the polymer is a naturally-occurring biopolymer. In some examples, the naturally-occurring biopolymer is selected from pullulan, trehalose, maltose, cellulose, dextran, and a combination of any thereof. In some examples, the naturally-occurring biopolymer is pullulan. In some examples, the polymer is a cyclic saccharide polymer or a derivative thereof. In some examples, the polymer is hydroxypropyl β-cyclodextrin. In some examples, the polymer is a synthetic polymer. In some examples, the synthetic polymer is selected from polystyrene, poly(meth)acrylate, and a combination of any thereof. In some examples, the synthetic polymer is a block copolymer comprising at least one poly(propylene oxide) block and at least one poly(ethylene oxide) block. In some examples, the synthetic polymer is a poloxamer.

In some examples, 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. In some examples, the composition further comprises a buffer selected from a phosphate buffer, tricine, and 2-(*N*-morpholino)ethanesulfonic acid. In some examples, the composition further comprises a surfactant selected from polysorbate 20, polysorbate 40, and polysorbate 80. In some examples, the composition comprises a reducing agent selected from thiourea and 6-aza-2-thiothymine. In some examples, the composition further comprises a salt selected from sodium chloride and sodium phosphate. In some examples, the composition further comprises a radical scavenger agent selected from ascorbic acid and sodium ascorbate. In some examples, the composition further comprises a chelating agent, and the chelating agent is selected from citric acid and trans-1,2-diaminocyclohexane-tetraacetic acid. In some examples, the composition further comprises a protein selected from bovine serum albumin, gelatin, and a polypeptide fraction of highly purified dermal collagen of porcine origin.

In some examples, the composition is in the form of a lyophilized powder or cake. In some examples, the composition is in the form of a malleable film. In some examples, the composition is a solution.

Provided herein are compositions comprising: a compound selected from coelenterazine and an analog or derivative thereof; and a surface selected from a paper or fiber matrix, a plastic, a glass, or a metal. 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 embodiments, the compound is JRW-

0238. In some examples, the compound is JRW-1743. In some examples, the compound is JRW-1744. In some examples, the composition further comprises a polymer. In some examples, the polymer is a naturally-occurring biopolymer. In some examples, the naturally-occurring biopolymer is selected from pullulan, trehalose, maltose, cellulose, dextran, and a combination of any thereof. In some examples, the naturally-occurring biopolymer is pullulan. In some examples, the polymer is a cyclic saccharide polymer or a derivative thereof. In some examples, the polymer is hydroxypropyl β-cyclodextrin. In some examples, the polymer is a synthetic polymer. In some examples, the synthetic polymer is selected from polystyrene, poly(meth)acrylate, and a combination of any thereof. In some examples, the synthetic polymer is a block copolymer comprising at least one poly(propylene oxide) block and at least one poly(ethylene oxide) block. In some examples, the synthetic polymer is a poloxamer.

In some examples, the composition further comprises a buffer, a surfactant, a reducing agent, a salt, a radical scavenger, a protein or any combination thereof. In some examples, the composition further comprises a buffer selected from a phosphate buffer, tricine, and 2-(*N*-morpholino)ethanesulfonic acid. In some examples, the composition further comprises a surfactant selected from polysorbate 20, polysorbate 40, and polysorbate 80. In some examples, the composition comprises a reducing agent selected from thiourea and 6-aza-2-thiothymine. In some examples, the composition further comprises a salt selected from sodium chloride and sodium phosphate. In some examples, the composition further comprises a radical scavenger agent selected from ascorbic acid and sodium ascorbate. In some examples, the composition further comprises a chelating agent, and the chelating agent is selected from citric acid and trans-1,2-diaminocyclohexane-tetraacetic acid. In some examples, the composition further comprises a protein selected from bovine serum albumin, gelatin, and a polypeptide fraction of highly purified dermal collagen of porcine origin. In some examples, the surface is selected from a cellulose paper, a nitrocellulose paper, a nylon paper, a cotton paper, a polyester paper, sodium carboxymethyl cellulose, a porous or polymeric membrane, a high purity cotton fiber, a cotton/rayon blended high purity cotton, and a glass microfiber.

Provided herein are methods of stabilizing a compound selected from coelenterazine and an analog or derivative thereof, comprising contacting the coelenterazine compound or the

analog or derivative thereof with an effective amount of a polymer and/or a paper or fiber matrix to form a composition. In one aspect, the present disclosure provides a method of stabilizing furimazine, comprising contacting the furimazine with an effective amount of pullulan to form a composition, wherein the compound is stabilized against thermal decomposition, chemical decomposition, light-induced decomposition, or any combination thereof. In some examples, the compound is stabilized against thermal decomposition, chemical decomposition, light-induced decomposition, or any combination thereof.

Provided herein are methods of improving the solubility of a compound selected from coelenterazine and an analog or derivative thereof, comprising contacting the coelenterazine compound or the analog or derivative thereof with an effective amount of a polymer and/or a paper or fiber matrix to form a composition. In some examples, the solubility of the compound is improved in an aqueous solution compared to the compound that has not been contacted with the polymer and/or the paper or fiber matrix. In one aspect, the present disclosure provides 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.

Provided herein are methods of improving the reconstitution rate of a compound selected from coelenterazine and an analog or derivative thereof comprising contacting the coelenterazine compound or the analog or derivative thereof with an effective amount of a polymer and/or a paper or fiber matrix to form a composition, wherein the reconstitution rate for the compound is improved compared to a compound that has not been contacted with the polymer or the paper or fiber matrix. In one aspect, the present disclosure provides 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. In another aspect, the present disclosure provides a kit comprising the composition as described herein.

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 some examples, the polymer is a naturally-occurring biopolymer. In some examples, the naturally-occurring biopolymer is selected from pullulan, trehalose, maltose, cellulose, dextran, and a combination of any thereof. In some examples, the naturally-occurring biopolymer is pullulan. In some examples, the polymer is a cyclic saccharide polymer or a derivative thereof. In some examples, the polymer is hydroxypropyl β-cyclodextrin. In some examples, the polymer is a synthetic polymer. In some examples, the synthetic polymer is selected from polystyrene, poly(meth)acrylate, and a combination of any thereof. In some examples, the synthetic polymer is a block copolymer comprising at least one poly(propylene oxide) block and at least one poly(ethylene oxide) block. In some examples, the synthetic polymer is a poloxamer.

In some examples, the composition further comprises a buffer, a surfactant, a reducing agent, a salt, a radical scavenger, a protein or any combination thereof. In some examples, the composition further comprises a buffer selected from a phosphate buffer, tricine, and 2-(*N*-morpholino)ethanesulfonic acid. In some examples, the composition further comprises a surfactant selected from polysorbate 20, polysorbate 40, and polysorbate 80. In some examples, the composition comprises a reducing agent selected from thiourea and 6-aza-2-thiothymine. In some examples, the composition further comprises a salt selected from sodium chloride and sodium phosphate. In some examples, the composition further comprises a radical scavenger agent selected from ascorbic acid and sodium ascorbate. In some examples, the composition further comprises a chelating agent, and the chelating agent is citric acid. In some examples, the composition further comprises a protein selected from bovine serum albumin, gelatin, and a polypeptide fraction of highly purified dermal collagen of porcine origin. In some examples, the paper or fiber matrix is selected from a cellulose paper, a nitrocellulose paper, a nylon paper, a cotton paper, a polyester paper, sodium carboxymethyl cellulose, a porous or polymeric membrane, a high purity cotton fiber, a cotton/rayon blended high purity cotton, and a glass microfiber.

In some examples, the contacting step comprises: dissolving the compound in an organic solvent to form a first solution; mixing the first solution with the polymer and/or the paper or fiber matrix to form a mixture; and drying the mixture. In some examples, the mixing step comprises dissolving the polymer in a second solution and mixing the second solution with the first solution. In some examples, the mixing step comprises applying the first solution to the paper or fiber matrix. In some examples, the drying step comprises lyophilization. In some examples, the drying step comprises air-drying. In some examples, the drying is conducted at ambient temperature in an inert atmosphere. In some examples, the drying comprises vacuum drying. In some examples, the drying is conducted at a temperature from about 30°C to about 70°C. In some examples, one or all of the solutions are deoxygenated.

In some examples, the method comprises contacting the compound with the polymer. In some examples, the method comprises contacting the polymer with the paper or fiber matrix. In some examples, the method comprises contacting the polymer with the polymer and the paper or fiber matrix.

Provided herein are kits comprising any one of the compositions disclosed herein. In some examples, the composition is included in one or more containers. In some examples, the composition is included in a plurality of tubes. In some examples, the composition is in the form of a plurality of paper spots, each spot having a diameter of about 2 mm to about 5 mm.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A-C show signal kinetics when compositions according to the present disclosure were tested for luminescence output in (A) phosphate buffered saline (PBS), pH 7.0 and (B) Nano-Glo® Luciferase Assay Buffer as described in Example 1. FIG. 1(C) shows images of furimazine substrate samples in pullulan-based lyophilized cake and pullulan film-droplet formulations.

FIGS. 2A-C show RLU values at various time points following addition of purified NanoLuc® enzyme when compositions according to the present disclosure were tested for luminescence output in PBS, pH 7.0 as described in Example 1.

FIGS. 3A-C show RLU values at various time points following addition of purified NanoLuc® enzyme when compositions according to the present disclosure were tested for luminescence output in Nano-Glo® Luciferase Assay Buffer as described in Example 1.

FIGS. 4A-C show absorbance values in aqueous solution when compositions according to the present disclosure were tested for absorbance over the range of 210-600 nm in PBS, pH 6.8 as described in Example 2.

FIG. 5 shows images demonstrating the ability of the compositions according to the present disclosure to reconstitute into PBS, pH 7.0 as described in Example 3.

FIGS. 6A-B show absorbance values over the range of 210-600nm of pullulan in PBS, pH 6.8 as described in Example 4.

FIGS. 7A-B show representative HPLC traces for 0% w/v pullulan-based lyophilized cake formulations containing furimazine at (A) 0 hours and (B) 5 hours after reconstitution as described in Example 5.

FIGS. 8A-B show representative HPLC traces for 2.5% w/v pullulan-based lyophilized cake formulations containing furimazine at (A) 0 hours and (B) 5 hours after reconstitution as described in Example 5.

CLAIMS

1. 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.

2. The composition of claim 1, wherein the compound is furimazine.

3. The composition of claim 1 or claim 2, wherein the naturally-occurring biopolymer is selected from pullulan, trehalose, maltose, cellulose, dextran, and a combination of any thereof.

4. The composition of claim 3, wherein the naturally-occurring biopolymer is pullulan.

5. The composition of any one of claims 1-4, 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.

6. The composition of claim 5, wherein the composition further comprises a buffer selected from a phosphate buffer, tricine, and 2-(*N*-morpholino)ethanesulfonic acid.

7. The composition of claim 5 or claim 6, wherein the composition further comprises a surfactant selected from polysorbate 20, polysorbate 40, and polysorbate 80.

8. The composition of any one of claims 5-7, wherein the composition comprises a reducing agent selected from thiourea and 6-aza-2-thiothymine.

9. The composition of any one of claims 5-8, wherein the composition further comprises a salt selected from sodium chloride and sodium phosphate.

10. The composition of any one of claims 5-9, wherein the composition further comprises a radical scavenger agent selected from ascorbic acid and sodium ascorbate.

11. The composition of any one of claims 5-10, wherein the composition further comprises a chelating agent, and the chelating agent selected from citric acid and trans-1,2-diaminocyclohexane-tetraacetic acid.

12. The composition of any one of claims 5-11, 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.

13. The composition of any one of claims 5-12, wherein the composition is in the form of a lyophilized powder or cake.

14. The composition of any one of claim 5-13, wherein the composition is in the form of a malleable film.

15. The composition of any one of claims 5-14, wherein the composition is a solution.

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

17. 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.

18. 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.

19. A kit comprising the composition of any one of claims 1-15.