Nos. WO97/36931; WO98/12539; WO99/14599; WO99/32662; WO99/58962; WO99/63347; WO00/03233 and WO98/57154.

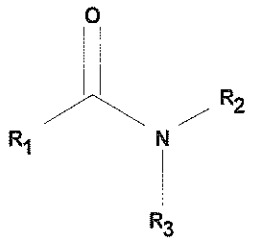
Commercially available ECL instruments have demonstrated exceptional performance. They have become widely used for reasons including their excellent sensitivity, dynamic range, precision, and tolerance of complex sample matrices. The commercially available instrumentation uses flow cell-based designs with permanent reusable flow cells.

Available sample volumes for the determination of analytes are often limited and more and more different analytes have to be determined out of one sample. Also the development of faster laboratory equipment for assay automation and more sensitive methods for the detection of analytes are required. This leads to the need for high sensitive and specific electrochemiluminescent assays and methods for performing them. In addition improvements associated with safety hazards or environmental concerns should be considered.

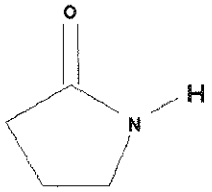
However, even more sensitive detection of analytes would be of great advantage. Thus, the object of the present invention was to improve said known methods and reagent compositions especially with respect to enhancement of the ECL signal and an improved analyte detection in combination with electrochemiluminescent procedures. It would be desirable to find novel signal enhancing reagents and/or compounds with improved performance in electrochemiluminescent assays.

Summary ~~of the invention~~

The present ~~invention~~disclosure in one ~~embodiment~~aspect concerns a method for measuring an analyte in a sample via electrochemiluminescent detection, comprising the steps of a) incubating the sample with a detection reagent labeled with an electrochemiluminescent group, b) separating analyte-bound and analyte-free labeled detection reagent, c) incubating the separated labeled detection reagent with a reagent composition comprising i) at least one coreactant, and ii) at least one compound selected from the group of carbonic acid amides of Formula I and Formula II,

Formula I 

with R_1 = CH_3, CH_2F, CH_2Cl, CH_2CH_3, $CHClCH_3$, CH_2CH_2Cl, $C(CH_3)_2CH_3$, $CH_2CH_2CH_3$, $CClHCH_2CH_3$ or $CH_2CH_2CH_2CH_3$,
with R_2 = H, and
with R_3 = H,

Formula II, 

d) electrochemically triggering the release of luminescence, and e) determining the electrochemiluminescence (ECL) signal thereby measuring the analyte.

The present ~~invention~~disclosure also concerns a reagent composition for determining ECL, comprising i) a compound selected from the group of carbonic acid amides of Formula I and Formula II and ii) at least one coreactant.

In another aspect, there is provided a reagent composition for determining ECL, comprising

i) a compound selected from the group of carbonic acid amides of Formula I and Formula II, and
ii) at least one coreactant,

wherein the coreactant is selected from the group consisting of tertiary amines (e.g. tripropylamine (TPA)), oxalate, and persulfate.

The present ~~invention~~disclosure further relates to a reagent mixture, comprising a reagent composition for detemining ECL, comprising i) a compound selected from the group of carbonic acid amides of Formula I and Formula II and ii) at least one coreactant, a sample to be investigated, and at least one detecting reagent labeled with an electrochemiluminescent group.

In another aspect, there is provided a reagent mixture for determining ECL, comprising a reagent composition as defined above, a sample to be investigated and at least one detection reagent labeled with an electrochemiluminescent group.

In another aspect, there is provided the use of a reagent composition as defined above in the determination of ECL.

In another aspect, there is provided the use of a carbonic acid amide selected from the group of Formula I and Formula II for performing an electrochemiluminescent detection procedure.

The present ~~invention~~disclosure also concerns a kit for measuring ECL, which contains a reagent composition for detemining ECL, comprising i) a compound selected from the group of carbonic acid amides of Formula I and Formula II and ii) at least one coreactant.

In another aspect, there is provided a kit for measuring ECL, which contains a reagent composition as defined above.

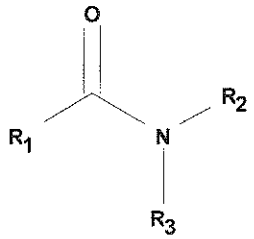
The ~~invention~~disclosure, as well as additional objects, features and advantages thereof, will be understood more fully from the following detailed description of certain preferred embodiments.

~~Enclosure to letter of August 23, 2012~~
~~International Application No. PCT/EP2011/068540~~
~~Applicant: Roche Diagnostics GmbH, et al.~~
~~Applicant's Ref.: 27067 WO-TH~~

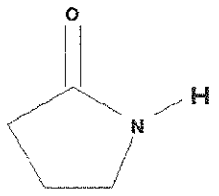
~~Amended~~ Claims

1. A method for measuring an analyte in a sample via electrochemiluminescent detection, comprising the steps of:

   a) incubating the sample with a detection reagent labeled with an electrochemiluminescent group,
   b) separating analyte-bound and analyte-free labeled detection reagent,
   c) incubating the separated labeled detection reagent with a reagent composition comprising
      i) at least one coreactant, and
      ii) at least one compound selected from the group of carbonic acid amides of Formula I and Formula II,

      Formula I 

      with R_1 = with R_1 = CH_3, CH_2F, CH_2Cl, CH_2CH_3, $CHClCH_3$, CH_2CH_2Cl, $C(CH_3)_2CH_3$, $CH_2CH_2CH_3$, $CClHCH_2CH_3$ or $CH_2CH_2CH_2CH_3$, with R_2 = H, and with R_3 = H,

      Formula II, 

   d) electrochemically triggering the release of luminescence, and
   e) determining the electrochemiluminescence (ECL) signal thereby measuring the analyte.

2. The method according to claim 1, characterized in that the measurement of an analyte in a sample using ECL is performed in an aqueous solution.

3. The method according to claims 1 or 2, characterized in that the carbonic acid amide is selected from the group consisting of acetamide, 2-fluoroacetamide, 2-chloroacteamide, propanamide, 2-chloropropanamide, 3-chloropropanamide, butanamide and 2-chlorobutanamide.

4. The method according to any of claims 1 to 3, characterized in that the reagent composition comprises a carbonic acid amide in a concentration of 0.01 M to 0.25 M.

5. The method according to any of claims 1 to 4, characterized in that the reagent composition of step 1c) comprises a preservative.

6. The method according to claim 5, characterized in that the reagent composition of step 1c) comprises a preservative in a concentration of 0.1% to 5%.

7. The method according to claims 5 or 6, characterized in that the reagent composition of step 1c) comprises a preservative selected from the group consisting of boric acid and borate.

8. The method according to any of claims 1 to 7, characterized in that the reagent composition of step 1c) comprises a detergent and a buffering agent.

9. The method according to claim 8, characterized in that the reagent composition of step 1c) comprises in addition a salt and/or an anti-foam agent.

10. A reagent composition for determining ECL, comprising

    i) a compound selected from the group of carbonic acid amides of Formula I and Formula II, and
    ii) at least one coreactant,

    wherein the coreactant is selected from the group consisting of tertiary amines (e.g. tripropylamine (TPA)), oxalate, and persulfate.

11. The reagent composition according to claim 10, characterized in that the reagent composition contains in adddition a preservative selected from the group consisting of boric acid and borate.

12. A reagent mixture for determining ECL, comprising a reagent composition according to claims 10 or 11, a sample to be investigated and at least one detection reagent labeled with an electrochemiluminescent group.

13. Use of a reagent composition according to claims 10 or 11 in the determination of ECL.

14. Use of a carbonic acid amide selected from the group of Formula I and Formula II for performing an electrochemiluminescent detection procedure.

15. A kit for measuring ECL, which contains a reagent composition according to claims 10 or 11.