Examiners' Report Paper A 2014 (Chemistry)

1. Introduction

In this paper, the applicant is a small biotech company working in the field of protein production. The company has a particular interest to provide said proteins in a solution in which they remain stable and thus biologically active over time.

Cholera is caused by the release of a protein belonging to the family of Bacterial Diarrhea Proteins (BDP). The family of BDP proteins comprises three types. Each of these three types consist of a single Alpha (A) and either two, four or five Beta (B) subunits (AB2, AB4 and AB5). AB5-type proteins are causative for diarrhea but their mechanism of action is unclear. Research on AB5-type proteins is difficult because of their poor stability in the standard diluent currently used.

The applicant developed a method, which, for the very first time, makes it possible to detect whether an AB5-type protein is in its complexed or in its disintegrated state, i.e. has fallen apart into its subunits. On this basis, the applicant set up a screening assay to search for stabilising solutions in which an AB5-type protein remains in its complexed and thus biologically active state. Two stabilising solutions were found.

The applicant expressed the wish to protect all other stabilising solutions that can be found using said screening method.

1.1. Prior art document D1

Prior art document D1 discloses a method that comprises the same chromatography and elution steps as the method put forward by the applicant to detect whether an AB5-type protein is in its complexed or in its desintegrated state. In D1, however, this method is used for an entirely different protein, i.e. VIP2. There is also no disclosure in D1 that this method has the potential to detect and quantify to which extent an AB5-type protein is in its complexed state or has disintegrated into its subunits.

1.2. Prior art document D2

Prior art document D2 is a patent application, which also deals with an AB5-type protein in the context of cholera. The aim of this disclosure is, however, to reduce the toxic effects of AB5-type proteins in order to improve their use in animal models.

In this document, there is a disclosure of a composition comprising a mutated AB5-type protein in one of the stabilising solutions identified by the screening method of the applicant (mutated AB5-type protein + PBS + 0.25% wt.% CHAPS).

1.3. Claims

A total of 70 marks is available for the independent claims. For dependent claims up to 15 marks can be awarded. Similarly, the description can attract 15 marks.
No marks were awarded for any claims subsequent to the 15th, claim since the client stated that claims fees will not be paid.

1.3.1 Independent process claim – “Proportion method”

A method for determining the proportion of an AB5-type BDP protein (or AB5-type protein) in its complexed state present in a sample characterised by the steps of:

(i) submitting a sample of an AB5-type BDP protein to column chromatography whereby the support material comprises hydroxylated polymethacrylate (HyPM) material with residual carboxyl groups,
(ii) eluting the proteins with a buffer comprising Tris-HCl 200mM and Na_2SO_4 100mM at a pH ranging from pH 6.8 to 7.6,
(iii) detecting the eluted protein, and
(iv) determining the amount of B5 and of AB5 protein in its complexed state to calculate the proportion of the AB5-type BDP in its complexed state.

A maximum of 15 marks can be awarded for this process claim directed to a method of detecting and measuring to which extent an AB5-type protein is present in its complexed state or has disintegrated into its subunits.

This claim is novel over the method disclosed in D1 due to the purpose feature which directs the method to AB5-type proteins and the determination of its integrity. The method is also inventive over the prior art as its ability to detect to which extent the AB5-type protein is in its complexed state or has disintegrated has not been suggested in the prior art.

Alternatively, it was possible to formulate a claim referring to a method of separating complexed AB5 from B5 of the AB5-type BDP protein (“Separation method”). This claim is novel and inventive for the reasons detailed above for the “Proportion method” and can also gain 15 marks. If the candidate chose this claim formulation, the feature describing the calculation to determine the extent to which an AB5-type protein is present in its complexed state or has disintegrated into its subunits becomes a useful dependent claim and should be claimed.
The candidates should realise that the pH range of 6.8-7.6, as specified in paragraph [010] of the client’s letter, could be claimed. A limited pH range led to a deduction of marks.

The candidates lost 5 marks when essential technical features were missing (e.g. buffer composition missing; no limitation to AB5-type proteins). The same deduction was made for unnecessary limitations (e.g. limitation to EcT or Cvt; inclusion of the particle size and porosity values of 6μm and 250nm; pH limited to 7.2; inclusion of the feature SF > 0.7; limitation to “natural” AB5–type protein). A limitation of this claim to HPLC led to a deduction of 3 marks. A minor lack of clarity led to a loss of 2 marks whereas a substantial lack of coherence of the claim was penalised with a deduction of 5 marks (e.g. method directed to the determination of the proportion but only AB5 or only B5 was quantified). A claim lacking novelty did not gain any marks.

1.3.2 Independent process claim – “Screening method”

The applicant searched for solutions that stabilise the AB5-type protein in its complexed state (stabilising solutions) using the above “Proportion method”. This screening method forms the basis for a further independent claim, which could have the following wording:

A method for identifying (or preparing) a solution that stabilizes an AB5-type BDP protein in its complexed state, the method comprises the steps of

(i) combining an AB5-type BDP protein with an aqueous candidate stabilising solution,
(ii) incubating the test sample of step (i),
(iii) analysing the test sample of step (ii) by the method defined in claim 1, and
(iv) calculating the stability factor (SF) as the ratio of the amount of AB5-type protein in its complexed state divided by the total amount of AB5 + B5, and whereas a SF exceeding 70% indicates that the candidate stabilizing solution stabilises the AB5-type BDP protein in its complexed state.

A maximum of 20 marks can be awarded for this independent claim.

The method must comprise the features defined as essential in the letter by the applicant. Of course, the claim must also comprise the elements of the "Proportion method" already claimed.

This claim is novel and inventive for the same reasons as the "Proportion method".

The candidates lost 5 marks when essential technical features were missing (e.g. no reference to the "proportion-method" in claim 1; no calculation of the stability factor (SF)). The same deduction was made for unnecessary limitations (e.g. limitation to CvT or EcT. A lack of clarity led to a loss of 2 marks (e.g. definition of SF is missing) whereas a claim lacking novelty did not gain any marks.

1.3.3 Independent composition and use claims – "Stabilising solution"

The following claims could be formulated:

A composition comprising an AB5-type BDP protein, a phosphate buffer and at least 10mM L-Arginine.

A composition comprising an AB5-type BDP protein, PBS and at least 0.05% wt.% CHAPS, the composition comprising an AB5-type BDP protein, PBS and 0.25% wt.% being excluded.

Use of a composition comprising a phosphate buffer and at least 10mM L-Arginine for stabilising an AB5-type BDP protein.

Use of a composition comprising PBS and at least 0.05% wt.% CHAPS for stabilising an AB5-type BDP protein.

To correctly formulate these claims, the candidates must consider the letter of the applicant and D2. They must realise that the candidate stabilising solutions tested in the client's letter were taken from commercially available kits. Any claim to a stabilising solution as such, i.e. without an AB5-type protein, therefore lacks novelty.

Among the candidate stabilising solutions tested by the applicant, two were found to have the desired activity, namely

a) AB5-type protein + Phosphate buffer + at least 10mM L-arginine and
b) AB5-type protein + PBS + 0.25% wt.% CHAPS.

Candidates should realise that stabilising solution b) has already been disclosed in D2. The fact that the stabilising effect has not been noticed in D2 is not a basis on which a product claim can be formulated. It is, however, possible to formulate a claim avoiding the disclosure of D2. For example, it is possible to exclude the only CHAPS concentration disclosed in D2, i.e. 0.25%. It is also possible to limit the CHAPS-based stabilizing solution to non-mutated, i.e. natural or wild-type, AB5-type protein as D2 only discloses a modified, i.e. mutated, variant of the AB5-type protein in this stabilising solution.

Candidates should also realise that only the specific compositions disclosed in the client's letter have the said stabilising effect. Omission of elements of the stabilising solutions such as the buffer results in the deduction of marks.

Moreover, it is also possible to formulate use claims referring to the use of the above stabilising compositions for stabilising of an AB5-type protein. Different to the composition claims, however, it was not necessary for the CHAPS-based use claim to exclude the specific composition disclosed in D2. These use claims are both novel and inventive over the prior art as there is no disclosure that the said compositions can be used for this purpose.

The applicant expressed in his letter the wish to also protect those stabilising solutions that could be found using the screening method described. The candidates should, however, realise that in this case such a claim to yet-unidentified solutions represents a "reach-through claim". In the present case, the data provided by the applicant demonstrate that it is unpredictable which candidate composition has the desired stabilising activity (see Table 2). This results in a lack of support and clarity for such a "reach-through claim". Moreover, as demonstrated by the data provided by the applicant, such a claim also encompasses stabilising solutions already known in the art such as the composition comprising PBS + 0.25 wt% CHAPS which is disclosed in D2.

20 marks can be awarded for each correct claim to a product and 15 marks for each correct use claim. Each product and use claim is marked individually. Overall, however, the candidate may be awarded a maximum of 35 marks for all of the product and use claims together. 15 marks are deducted for "reach-through claims" and product claims to the stabilizing solution as such (i.e. not comprising the protein) as these were commercially available and hence not novel.

The candidates lost 5 marks when essential technical features were missing (e.g. "at least 10mM" for arginine; "at least 0.05 wt.%" for CHAPS) in the claims. The same deduction was made for unnecessary limitations (e.g. including "pH 7.4" for the buffer of the L-arginine composition). A lack of clarity led to a loss of 2 marks and a claim lacking novelty did not gain any marks.

2. Dependent claims

A total of 15 marks are available for the dependent claims. The following claims could give realistic fallback positions:

- For the separation method:
    - i) pH 7.2
    - ii) particle size of 6μm and porosity of 250nm of the column material used
    - iii) use of an HPLC column
    - iv) incorporation of a step of calculating the Stability Factor (SF)
- For the screening method:
    - v) incubation step at room temperature
    - vi) incubation step at 4°C
    - vii) keeping the sample during incubation static condition
    - viii) shaking the sample during incubation
    - ix) testing whether stabilised AB5-type BDP protein is biologically active
- For the composition claims:
    - x) concentrations of L-arginine up to 50mM
    - xi) concentrations of CHAPS of 0.15 wt.% or 0.35 wt.%
    - xii) specifying the AB5-type protein to be EcT or CvT
- For the use claims:
    - xiii) concentrations of L-arginine up to 50mM
    - xiv) concentrations of CHAPS of 0.15 wt.%, 0.25 wt.% or 0.35 wt.%
    - xv) specifying the AB5-type protein to be EcT or CvT

Dependent claims could attract up to 2 marks each but not exceeding a total of 15 marks.

3. Description

Up to 15 marks are available for the description. 4 marks are available for a summary of the relevant aspects of prior art documents D1 and D2. It was also expected that, as required by Rule 42(1)(c) EPC, the technical problem and its solution are described. 6 marks are available for this aspect. Finally, consistency between the claims and the description was evaluated (5 marks).

Example set of claims:

1. A method for determining the proportion of an AB5-type BDP protein (or AB5-type protein) in its complexed state present in a sample characterised by the steps of

   (i) submitting a sample of an AB5-type BDP protein to column chromatography whereby the support material comprises hydroxylated polymethacrylate (HyPM) material with residual carboxyl groups,

   (ii) eluting the proteins with a buffer comprising Tris-HCl 200mM and Na_2SO_4 100mM at a pH ranging from pH 6.8 to 7.6,

   (iii) detecting the eluted protein, and

   (iv) determining the amount of B5 and of AB5 protein in its complexed state to calculate the proportion of the AB5-type BDP in its complexed state.

2. The method according to claim 1, wherein the pH is 7.2.

3. The method according to claims 1 or 2 wherein the HyPM has a particle size of 6μm and a porosity of 250nm.

4. The method according to claims 1-3 wherein chromatography is performed in the format of a High-Performance-Liquid-Chromatography.

5. A method for identifying (or preparing) a solution that stabilizes an AB5-type BDP protein in its complexed state, the method comprises the steps of

   (i) combining an AB5-type BDP protein with an aqueous candidate stabilising solution,

   (ii) incubating the test sample of step (i),

   (iii) analysing the test sample of step (ii) by the method defined in any of claims 1-4, and

   (iv) calculating the stability factor (SF) as the ratio of the amount of AB5-type protein in its complexed state divided by the total amount of AB5 + B5,

   whereas a SF exceeding 70% indicates that the candidate stabilizing solution stabilises the AB5-type BDP protein in its complexed state.

6 The method according to claim 5 wherein the sample is maintained at room temperature.

7. The method of any of claims 5 or 6 in which the sample is maintained in a static condition during incubation.

8. The method of any of claims 5-6 in which the sample is shaken during incubation.

9. The method of any of claims 5-8 further comprising the step of testing whether the stabilized AB5-type BDP protein is biologically active.

10. A composition comprising an AB5-type BDP protein, a phosphate buffer and at least 10mM L-Arginine.

11. The composition according to claim 10 wherein the AB5-type protein is EcT or CvT.

12. Use of a composition comprising a phosphate buffer and at least 10mM L-Arginine for stabilising an AB5-type BDP protein.

13. Use of a composition comprising PBS and at least 0.05 wt.% CHAPS for stabilising an AB5-type BDP protein.

14. The use according to claim 13, wherein CHAPS is present in an amount of 0.15 wt.%, 0.25 wt.% or 0.35 wt.%.

15. The use according to claims 12-14 wherein the AB5-type protein is EcT or CvT.

EXAMINATION COMMITTEE I

Candidate No. _____

Paper A (Chemistry) 2014 - Marking Sheet

Category		Maximum possible	Marks awarded	
Independent claims	Method for determining the proportion complexed versus non-complexed	15		
	Method for identifying a stabilizing solution	20		
	Composition and/or uses	35		
Dependent claims		15		
Description		15		
Total		100		

Examination Committee I agrees on ..... marks and recommends the following grade to the Examination Board:

☐ PASS (50-100)

☐ COMPENSABLE FAIL (45-49)

☐ FAIL (0-44)

24 June 2014

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Chairman of Examination Committee I