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Development of a novel platform of virus-like particle (VLP) based vaccine against coronavirus 2019 (SARS-CoV-2) by exposing of epitopes: an immunoinformatics approach

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Abstract

The emergence of a rapidly spreading and highly infectious COVID-19 outbreak by a novel coronavirus, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), has caused a global pandemic with unprecedented, social and economic dimensions. Therefore, the development of effective strategies is urgent to control COVID-19 outbreak. According to the recent investigations, cell entry of coronaviruses relies on binding of the viral spike glycoprotein to the host cellular receptors. Therefore, in the present study aimed to predict immunogenic epitopes in silico by analyzed spike protein. In parallel, by screening the immunogenic SARS-CoV-2 spike derived epitopes provided in the literature, we chose a set of epitopes believed to induce immunogenic response. Next, we provided the selected epitopes from both approaches, we performed immunoinformatic analysis that map identically to antigen regions and have antigenic properties. Finally, by suggesting a screened set of epitopes, we designed a novel virus-like particle (VLP) vaccine, optimized to be produced in plants by using molecular farming biotechnology techniques. We anticipate our assay to be a starting point for guiding experimental efforts toward the development of a vaccine against SARS-CoV-2.

KEYWORDS

COVID-19, Severe Acute Respiratory Syndrome Coronavirus 2, Epitope Prediction, Virus-Like Particles, Vaccine, Immunoinformatics.

INTRODUCTION

Coronaviruses are positive-sense single-stranded RNA viruses that belong to the family Coronaviridae [1]. They mainly infect animals, including birds and mammals. In humans, they generally cause mild respiratory infections [1]. However, some recent human coronavirus
infections have resulted in lethal endemics, which include the SARS-CoV (Severe Acute Respiratory Syndrome), MERS-CoV (Middle East Respiratory Syndrome) and the newly emerged SARS-CoV-2 [2]. By phylogenetical analysis of the full-length genome, research groups are suggesting that SARS-CoV-2 is quite similar to SARS-CoV [3]. Moreover, they putatively have similar cell entry mechanisms and human cell receptors usage [4]. SARS-CoV-2 has a positive-sense single-stranded RNA in size of ~30 kilobase, which like other coronaviruses, encodes for multiple structural and non-structural proteins [5]. The structural proteins include the spike (S) protein, the envelope (E) protein, the membrane (M) protein, and the nucleocapsid (N) protein [6].

Therapeutic targets will become available by gaining insights into cell entry mechanisms and viral transmission of SARS-CoV. The spike protein of SARS-CoV and MERS-CoV plays a vital role in receptor binding and membrane fusion [7]. Considering the high genetic similarity of the S protein between SARS-CoV and SARS-CoV2, we could conclude that S protein would have such a vital role in COVID-19 infection. In the recent findings, it has been reported that cell entry of SARS-CoV-2 depends on the binding of the viral S proteins to cellular receptors and on S protein priming by host cell proteases [8]. SARS-CoV-2 binds with a high affinity to human Angiotensin-Converting Enzyme 2 (ACE2) and uses it as an entry receptor to invade target cells [1]. Therefore, the spike protein of SARS-CoV is anticipated to be an important component of candidate vaccines [9] and to inhibit the spike-mediated entry by SARS-CoV-2 it is vital to consider spike protein of SARS-CoV-2 as one of the most important targets to develop a vaccine or treatment to cure COVID-19 infection. Recent studies showed that interleukin 6 receptor antagonist therapy and targeting the toll-like receptor 5 are the purposes for control COVID-19 [10-12].
Currently, limited immunological information, such as probable immunogenic epitopes eliciting antibodies or T cell responses, are available related to SARS-CoV-2. Adaptive immunity mediated by T- and B-cells, is capable of developing pathogen-specific memory that confers immunological protection [13]. In particular, the B- and T-cells recognize portions known as epitopes within their cognate antigens. The identification of epitopes in antigens is of great interest for a better understanding of disease etiology and monitoring the immune system during the infection, which can result in designing diagnostics assays and possibly epitope-based vaccines specific for the occurring infection. Epitope identification is costly and time-consuming, as it requires experimental screening of large arrays of potential epitope candidates. Fortunately, researchers have developed \textit{in silico} prediction methods that have significantly increased the simplicity of epitope mapping by decreasing the list of potential epitope candidates for experimental testing.

The main information that would help us to choose desirable epitopes is to figure out which parts of the SARS-CoV-2 sequence are recognized by human immune responses. Such data and information are unfortunately currently very limited since a short time is passed since the discovery of the SARS-CoV-2 virus. Gaining this information would facilitate the evaluation of vaccine candidate immunogenicity, as well as monitoring of the potential consequences of mutational events and epitope escape as the virus is transmitted through human populations [14].

Another approach to map and suggest potential epitopes for designing a vaccine against SARS-CoV-2 is to select them from provided epitopes in the previous researches. In a recent study, researchers have determined the crystal structure of the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein in complex with CR3022, a neutralizing antibody previously isolated from a convalescent SARS patient [15]. A highly conserved epitope is the target of CR3022 that
enables cross-reactive binding between SARS-CoV-2 and SARS-CoV. Therefore, among the
previously reported anti-SARS CoV antibodies, we considered CR3022 to be one of the most
promising ones to choose our desired epitopes from its sequence. The epitope of CR3022 does
not overlap with the ACE2 binding site within SARS-CoV-2 RBD [15].

Virus-like particles (VLPs) have gradually emerged as vaccine delivery agents that are
spontaneously assembled from viral structural proteins [16]. These are multimeric structures that
can directly stimulate immune cells by mimicking the three-dimensional conformation of native
viruses. Moreover, VLPs are devoid of infectious genetic material which makes them inherently
safer than attenuated or inactivated virus preparations [17]. They contain functional viral proteins
responsible for cell penetration by the virus, therefore efficient cell entry and thus tissue-specific
targeting, determined by the origin of the virus is gained using them as a vaccine. VLP vaccines
provide delivery systems that combine good safety profiles with strong immunogenicity and thus
are safe alternatives to inactivated infectious viruses. VLPs have excellent adjuvant properties
and are capable of inducing innate and adaptive immune responses. They present both, high-
density B-cell epitopes, for antibody production and intracellular T-cell epitopes, thus inducing,
respectively, potent humoral and cellular immune responses [18]. Experience with previously
introduced VLP based vaccines demonstrates that the uptake of VLPs by antigen-presenting cells
can trigger efficient immune responses which result in the control of infection [19]. These
features make VLPs a top platform for the making of a safe and effective vaccine [20]. VLPs are
produced using different expression systems, such as bacterial, yeast, mammalian, plant, or
insect cells. For the presently designed vaccine by our research team, we suggest plants as an
expression system. VLPs derived from Hepatitis B virus (HBV) are the most studied chimeric
VLPs for epitope display [17] and the HBV is used to produce different vaccines [21].
By screening the determined SARS-CoV-2 derived epitopes in the immunogenic structural proteins of SARS-CoV-2 and using bioinformatics tools we suggested a list of potential epitopes for vaccine design. Our findings will potentially narrow down the quest for powerful targets for an effective peptide derived SARS-CoV-2 vaccine and help to direct development-focused studies to achieve a vaccine for COVID-19 infection as soon as possible.

MATERIALS AND METHODS

Literature review
Recent publications related to SARS-COV and SARS-COV2 epitopes were reviewed and the most reliable were selected to choose epitopes [1, 14, 22]. Our main focus and concern to choose epitopes from the researcher’s suggestions was their immunogenicity. Potential immunogenic peptides from SARS-COV2 for vaccine targets were selected by screening the most reliable reported conducted since the COVID-19 outbreak was started in early 2020. Epitopes that were more mentioned and repeated in different studies were selected and analyzed carefully.

Bioinformatics approaches, Data collection and pre-processing
Nucleotide sequences data of whole genome SARS-CoV-2 isolates accessible until April 2020 were downloaded from National Center for Biotechnology Information and GISAID database. Data with accession numbers MN95262 (China), MT281530 (Iran), EPIISL415460 (Netherlands), EPIISL417413 (Turkey), EPIISL417444 (Pakistan), EPIISL413515 (South Korea), EPIISL418411 (Finland), MT007544 (Australia), MN997409 (USA), EPIISL420890 (Japan) for SARS-CoV-2 NC004718 (SARS) and KY417142 (Bat SARS-like) were reported from different countries. For comparison of S proteins, nucleotide sequences were aligned using the ClustalW method implemented in Geneious Prime 2019 software.

Identification of epitopes and antigenicity analysis
Antigenic regions of S protein SARS-CoV-2 were determined using three tools; Geneious Prime 2019 software based on the EMBOSS 6.5.7 tools antigenic, CLC Genomics workbench 12 with Welling and Kolaskar-Tongaonkar scales and the Immune Epitope Database (IEDB). Antigenic regions were annotated in S protein amino acid sequences using Geneious Prime 2019 software for better comparison. Then epitopes were analyzed for binding to a specific MHC class I and class II molecules using IEDB.

The third protein structure of S protein was modeled and epitopes were validated using SWISS-MODEL tools and RCSB PDB (http://www.rcsb.org/structure/6VXX). The location of epitopes was determined on the third protein structure of S protein. Five epitopes from the literature review and five epitopes from bioinformatics tools were selected as the best candidates.

**Vaccine construction, modeling, and validation**

VLP of HBV core particles (HBc) was used as a platform for exposing SARS-CoV-2 epitopes in the major immunodominant region (MIR) region of HBc. The final production of this vaccine was predicted using SWISS-MODEL tools.

**Analysis of the physicochemical properties of suggested epitopes**

In order to characterize the physicochemical parameters of the introduced epitopes, we used http://web.expasy.org/protparam server. The calculated parameters include; molecular weight, extinction coefficient, theoretical pI, aliphatic index and grand average of hydropathicity.

**Allergenicity evaluation**

The allergenicity of the predicted peptides was computed by AllerCatPro server at (https://allercatpro.bii.a-star.edu.sg/). Accuracy of the AllerCatPro server is 84% compared with other prediction methods which range from 51 to 73%. At the highest sensitivity needed for
conservative assessments, AllerCatPro enhances specificity by 37-fold compared with the FAO/WHO rules [23].

RESULT/DISCUSSION

Collection of targeted protein sequence

By spreading SARS-CoV-2 and the epidemic of COVID-19 in early 2020, there is an urgent need for designing a suitable peptide vaccine component against the SARS-CoV-2. Currently, no approved specific antiviral vaccine targeting the SARS-CoV-2 virus, although some drugs are still under investigation. As the first attempt to manage this global concern scientists compared SARS-CoV-2 with previous coronaviruses. Results of the current investigations have revealed important commonalities between SARS-CoV-2 and SARS-CoV infection and have led to identifying potential targets for antiviral intervention [7]. According to phylogenetic analysis, high genetic similarity has been observed between SARS-CoV-2 and SARS-CoV [3]. Also, complete genomes of different isolates of SARS-CoV-2 have been released, and the latest published information on these genomes are provided [24].

In this study, we report for the first time the comparison of the nucleotide sequences of an Iranian isolate of SARS-CoV-2 with other countries including; China, Netherlands, Japan, Turkey, Pakistan, South Korea, Finland, Australia and the USA. Our analysis showed that S protein is conserved between isolates from different countries with 100% and 99% similarity (Table1). We also compared all of the above SARS-CoV-2 isolates with Bat SARS-like and SARS viruses, which showed about 72% and 75% similarity with SARS and Bat SARS-like, respectively. The high similarity of S protein of SARS-CoV-2 in different isolates proves that this protein and suggested epitopes acquired from it can be potentially best targets to model
vaccines against COVID-19. Also, a study showed that the S gene is more conserved about variations in the viral population in the patients [25].

Whilst the M and E proteins of SARS-CoV-2 are involved in virus packaging, the spike protein is the prominent intermediate of viral entry [26]. Both full-length S protein and its antigenic fragments, including the S1 subunit, NTD, RBD and S2 subunit, have been reported as potential targets for the development of subunit vaccines [27]. This information along with the observation of scientists that many epitopes located on the spike protein of SARS-CoV-2 are highly conserved\(^7\) and similar to SARS-CoV is very important and beneficial to us to consider this region for designing a VLP-based vaccine against SARS-CoV-2.

**Identification of epitopes**

Considering the high genetic similarity between SARS-CoV-2 and SARS-CoV, we focused particularly on the epitopes in the S proteins due to their dominant and long-lasting immune response previously reported against SARS-CoV [28] and their cell entry role. In recent studies, the S protein of SARS-CoV-2 has been considered as the target of vaccine development and even finding therapeutic medications. Due to the mentioned importance of the spike glycoprotein, this region of SARS-CoV-2 was analyzed for epitope identification in the IEDB server. Subsequently, the sequence of spike protein was also analyzed in Geneious 2019 and CLC Genomic workbench 12 software servers for the identification of the T-cell epitope that can combine with MHC-I and MHC-II molecules (Supplementary data 1). In order to identify crucial epitopes for designing an effective vaccine, the protein sequence of spike glycoprotein was explored thoroughly using multiple immunoinformatic-based servers and software.

Using immunoinformatics, we could recognize and characterize potential epitopes for the generation of a novel plant-derived VLP-based vaccine [29]. The IEDB is a source of epitope-
related information from the scientific literature in the context of infectious disease, allergy, and autoimmunity [30]. The IEDB provides bioinformatics tools and algorithms for analysis of epitope data and prediction of potential epitopes from novel sequences (Fig 1).

A key element in protection against coronaviruses is the neutralizing antibody response. Notably, a previously reported SARS-CoV RBD-specific human neutralizing mAb called CR3022 has been recently noticed to be able to bind to SARS-CoV-2 RBD with high affinity. Evidently, CR3022 recognizes an epitope on the RBD that does not overlap with the ACE2-binding site [22]. Therefore, analysis of the region of the previously introduced CR3022 mAb was also performed in the SARS-CoV2 S protein sequence, as a strategy to obtain the maximum reliable epitopes possible. Additionally, we analyzed the reported spike protein sequence that binds to ACE2, because receptor-binding domains of SARS-CoV-2 spike binding to human ACE2 correlates with the efficient spread of SARS-CoV-2 among humans. This region of 223aa on the spike protein is believed to have a critical role in the binding of SARS-CoV-2 to human host cells. Overall, by a multi-step computational approach, we predicted various antigenic regions within the S protein of CoV-2 that concur with our 5 identified epitopes as mentioned in Table 2.

In another approach, we identified multiple specific regions in SARS-CoV-2 spike protein that are ideal candidates as epitopes for vaccine design according to recent studies conducted on SARS-CoV-2, which resulted in finding another five specific epitopes (Table 2, candidate epitopes of S protein for exposing on Virus-like particles).

Having a high sequence similarity with immunogenic peptides but with greater predicted immunogenicity score our predicted epitopes can be used to build a peptide vaccine (Fig 2).

Also, these epitopes can be used as diagnostic tools for the SARS-CoV-2. We additionally
incorporated the information about the associated MHC alleles to provide a list of epitopes that seek to maximize population coverage globally (Supplementary data 1).

**Validation of epitopes**

For validation of selected epitopes, we used SWISS-MODEL Tools. The third structure of S protein was predicted and the position of epitopes on the protein structure was determined (Fig 3). This result confirmed that epitopes were selected from positions that can interact with host proteins such as antibodies. In order to be more confident the S protein model and epitopes were checked using RCSB PDB and it showed result similar SWISSMODEL.

**Vaccine construction, modeling, and validation**

Finally, the vaccine component was modeled using SWISS-MODEL tools. Our results obtained indicate that the designed vaccine model is in good quality (Fig 4). We predicted the VLP of HBc exposing epitopes of SARS-CoV-2 (Fig 4). HBc is the best choice as a platform for commercial vaccine production, not only against HBV but also against many other viral diseases. HBc showed high immunogenicity and enhanced presentation to the immune system. This VLP was expressed using an ORF and has the flexibility to allow a wide variety of foreign insertions without affecting the protein self-assembly and VLP function [31, 32]. This result confirmed that the inserting of the epitope in the HBc MIR region can expose these epitopes on the surface of VLP. The present study indicates the effectiveness of selected epitopes within the spike glycoprotein of SARS-CoV-2. These epitopes can be used to make an immunogenic multi-epitopic peptide vaccine against SARS-CoV-2. For better exposing of epitopes on VLP, they need to link by a peptide linker (Linker Database - IBIVU). (EAAAK)3 linker was suggested that easily fused with the virus coat protein [33].
Physical and chemical properties

Using the PROTPARAM tool the physical and chemical properties of epitopes were identified (Table 3, Chemo-physical properties of SARS-COV-2 epitopes). According to the result, the A1 epitope has the highest theoretical PI. Moreover, most peptides seem to remain stable regarding their instability index less than 40. The stability index is calculated based on the N-terminal ubiquitin-mediated degradation. Among all, E30, E24, A9 and A7 showed the highest thermo-stability due to their aliphatic index. Considering the GRAVY, the negative index indicates that the peptide is hydrophilic and soluble in aqueous solutions [34]. A2, E13, E29 and A1 showed to have the highest potential to interact with water molecules since they showed the least GRAVY.

Overall, a number of epitopes showed to serve as efficient vaccines against SARS-CoV-2 infection. However, E29 is predicted to be one of the most promising epitopes, since it has high thermo stability and is strongly hydrophilic.

Allergenicity evaluation

Allergen peptides or proteins stimulate an IgE antibody response [35]. The predicted vaccine candidate epitopes should not result in an allergic response to the body. Based on the prediction of the AllerCatPro server, all predicted epitopes are non-allergen.

CONCLUSION

We attempted to find various epitopes against SARS-CoV-2 by using immunoinformatic tools because quick identification of these epitopes is crucial to design vaccine component against COVID-19. Spike protein of SARS-CoV-2, which is currently believed to have the most important role in the binding and entry of these viruses to human cells, was analyzed to find epitopes. We chose five optimal epitopes from the literature and our parallel bioinformatics predictions identified five more epitopes for SARS-CoV-2. These epitopes are the ideal
candidate to formulate a multi‐epitopic peptide vaccine, not only because of being selected from
the ACE2 binding site and CR3022 antibody epitopic regions of spike protein but also because
their antigenic property was confirmed by using bioinformatics tools. Based on the selected
epitopes, we designed a vaccine to be generated in a short time in the near future. Our novel
platform of VLP based vaccine is designed to target the immune response toward these
conserved epitope regions which is potent to generate immunity against SARS-CoV-2.

Next, the significance and effectiveness of our suggested epitopes as an ideal vaccine candidate
against SARS-CoV-2 will be tested in plant‐derived VLPs. These immunoinformatic analyses
require several in vitro and in vivo validations before designing the vaccine to resist COVID-19.

The independent identification of the same regions using two approaches reflects the high
probability that our suggested epitopes are promising targets for immune recognition of SARS-
CoV-2. In conclusion, the use of available information related to SARS-CoV and SARS-CoV-2
epitopes in conjunction with bioinformatics predictions points to specific regions of SARS-CoV-
2 that have a high likelihood of being recognized by human immune responses.

Fig legends

Fig 1. The antigenic regions of S protein that were predicted by Immune Epitope Database
(IEDB).

Fig 2. Graphical display of the binding sites and antigenic regions of the SARS-CoV-2 spike
protein. Yellow; Epitopes recommended by articles, brown; antigenic regions predicted by
Genius 2019 software, blue; antigenic regions predicted by the CLC Genomic workbench 12
software and pink; predicted epitopes.

Fig 3. The third structure of S protein was determined using SWISS-MODEL and the position of
the epitopes on the protein structure are shown in orange.
Fig 4. General design of the VLP on hepatitis B core virus. A. The proposed structure is based on the PVX virus along with the VLP of the HBc virus and the epitopes, B. The final design of the hepatitis virus and the epitopes displayed in orange on the body of the virus. C. The predicted model of the third structure of the HBc along with the epitopes shown in orange.

Supplementary data 1. The list of MHC-I and II alleles that binding to epitopes.

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Table 1. Identity matrix of S protein using different isolate of SARS-CoV-2 and Bat SARS-like and SARS.

|          | China | Iran | Netherlands | Japan | Turkey | Pakistan | South Korea | Finland | Australia | USA | Bat SARS-like | SAR S |
|----------|-------|------|-------------|-------|--------|----------|-------------|---------|------------|-----|---------------|-------|
| China    | 100   | 100  | 100         | 99.9  | 100    | 100      | 100         | 100     | 100        | 100 | 75.9          | 72.2  |
| Iran     | 100   | 100  | 100         | 99.9  | 100    | 100      | 100         | 100     | 100        | 100 | 75.9          | 72.2  |
| Netherlands | 100  | 100  | 100         | 99.9  | 100    | 100      | 100         | 99.9    | 100        | 100 | 75.9          | 72.2  |
| Japan    | 100   | 100  | 100         | 99.9  | 100    | 100      | 100         | 99.9    | 100        | 100 | 75.9          | 72.2  |
| Turkey   | 99.9  | 99.9 | 99.9        | 100   | 100    | 100      | 99.9        | 99.9    | 99.9       | 100 | 75.9          | 72.2  |
| Pakistan | 100   | 100  | 100         | 100   | 100    | 100      | 100         | 100     | 100        | 100 | 75.9          | 72.2  |
| South Korea | 100  | 100  | 100         | 100   | 100    | 100      | 100         | 100     | 100        | 100 | 75.9          | 72.2  |
| Finland  | 100   | 100  | 100         | 100   | 100    | 100      | 100         | 100     | 100        | 100 | 75.9          | 72.2  |
| Australia| 100   | 100  | 100         | 99.9  | 100    | 100      | 100         | 100     | 100        | 100 | 75.9          | 72.2  |
| USA      | 100   | 100  | 100         | 100   | 100    | 100      | 100         | 100     | 100        | 100 | 75.9          | 72.2  |
| Bat SARS-like | 75.9 | 75.9 | 75.9        | 75.9  | 75.9   | 75.9     | 75.9        | 75.9    | 75.9       | 75.9 | 75.9          | 71.8  |
| SARS     | 72.2  | 72.2 | 72.2        | 72.2  | 72.2   | 72.2     | 72.2        | 72.2    | 72.2       | 72.2 | 72.2          | 71.8  |

Table 2. Candidate linear epitopes of S protein for exposing on Virus like particles.

| NAME | SEQUENCE | REFERENCE |
|------|----------|-----------|
| A1   | IHVSGTNGTKRF | -         |
| A2   | YYHKNNKSWMESEFRVYSS | -         |
| A3   | AGSTPCNGVEGFNC | -         |
| A7   | FGAISSVLNDILSRLDKV | -         |
| A9   | DVVIGIVNNTV | -         |
| E13  | FSQILPDPSKPSKRSFIE | Grifoni et al., (2020) |
| E21  | GAALQIPFAMQAMAYRF | Ahmed et al., (2020) |
| E24  | FIAGLIAIV | Ahmed et al., (2020) |
| E29  | RLNEVAKNRL | Ahmed et al., (2020) |
| E30  | VLNLDILSRL | Ahmed et al., (2020) |

Table 3. Chemo-physical properties of SARS-COV-2 epitopes.

| Epitope | Molecular | Theoretical | Extinction | Instability | Aliphatic | Grand average of |
|---------|-----------|-------------|------------|-------------|-----------|-----------------|
|    | weight  | pI   | coefficients | index | index | hydropathicity (GRAVY) |
|----|---------|------|--------------|-------|-------|------------------------|
| A1 | 1316.48 | 11.00| Not visible by UV spectrophotometry | 7.42  | 56.67 | -0.550                 |
| A2 | 2455.69 | 8.39 | 9970         | 86.29 | 15.26 | -1.505                 |
| A3 | 1355.46 | 4.00 | 125          | -8.89 | 27.86 | -0.071                 |
| A7 | 1947.26 | 5.96 | Not visible by UV spectrophotometry | 27.39 | 146.11 | 0.650                 |
| A9 | 1142.32 | 3.80 | Not visible by UV spectrophotometry | -14.06 | 176.36 | 1.291                 |
| E13| 2076.38 | 8.59 | Not visible by UV spectrophotometry | 121.50 | 65.00 | -0.689                 |
| E21| 1815.18 | 8.75 | 1490         | 27.11 | 73.75 | 0.631                 |
| E24| 916.17  | 5.52 | Not visible by UV spectrophotometry | -0.54 | 227.78 | 3.056                 |
| E29| 1056.23 | 8.75 | Not visible by UV spectrophotometry | 8.89  | 130.00 | -0.589                 |
| E30| 1042.24 | 5.81 | Not visible by UV spectrophotometry | 51.69 | 205.56 | 0.867                 |
