

# In-vitro Studies with Artificial Saliva: An Up-to Date Overview of the Literature

## SUMMARY

*Artificial saliva (AS) is an aqueous solution designed to mimic the properties of natural saliva and is widely used in various in vitro studies. This investigation aimed to identify the most frequently selected AS compositions across different research categories. A systematic search was conducted using Web of Science, PubMed, and Scopus databases, focusing on articles published in the last decade with the keywords “artificial saliva” and “in vitro studies.” A total of 569 articles were reviewed and categorized based on research objectives: (a) dental tissue behavior and biocompatibility, (b) micromechanical surface properties, (c) chemical changes in materials, (d) food-drug interactions, and (e) microbiological and biofilm studies. AS compositions were grouped and analyzed using the Bonferroni test for frequency comparison. Salt-based solutions were the most used (42%), followed by formulations containing both salt and urea (20.2%). Despite widespread use, many studies lacked detailed descriptions of AS composition or preparation methods, complicating reproducibility. Furthermore, standardized protocols for AS formulations are absent, and specific chemical compositions tailored to each research category remain underdefined. Future research is essential to establish optimal AS formulations for distinct experimental contexts and improve consistency across studies.*

**Keywords:** Artificial saliva, In vitro studies, composition, Dental tissues, Biocompatibility, Micromechanical properties, Changes in chemical properties Food-Drug studies, Biofilm studies, microbiological study.

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## Introduction

Natural saliva, which is produced by the major and minor salivary glands within the oral cavity, performs various functions. It is essential for maintaining oral cavity homeostasis<sup>1</sup>. It assists in the prevention of oral cavity infections and the remineralization of dental tissues. Saliva is employed to detect flavors, as the substances dissolved in the saliva and activate the taste buds on the tongue. Natural saliva, which contains mucus, enzymes, and other proteins, protects the mucous membrane during chewing or speaking by regulating the pH and acting as a lubricant<sup>2</sup>. The microbes inhabiting

natural saliva continuously alter their composition, resulting in its rapid inactivity outside the mouth<sup>1</sup>.

An aqueous medium known as artificial saliva (AS) is frequently employed to replicate the properties of natural saliva in various applications. “Substitution therapy” is one of these applications for patients with dry mouth<sup>3</sup>. The remaining applications of AS are research-based. Researchers have created diverse AS compositions to investigate the properties of dental tissues, assess the toxicity of substances and drugs, evaluate the quality of dental materials, and investigate microbial behavior, biofilm growth, and other topics<sup>4-7</sup>. It is also feasible to generate cavities that resemble the original enamel lesion

through the utilization of a cariogenic environment, a bacterial culture, and AS<sup>7</sup>.

The inability of AS to replicate all the properties of natural saliva is one of its drawbacks<sup>8</sup>. First, the AS is more stable than the dynamic environment of the oral cavity; its consistent pH and temperature, which prevents it from undergoing the same variations<sup>9</sup>. Furthermore, AS lacks the diverse array of glycoproteins and enzymes that are present in natural saliva<sup>10</sup>. Natural saliva also contains microbes, such as bacteria and fungi. It is possible that these microbes could interact with some materials or chemical bonds within AS. Other reviews have focused exclusively on dentist specific applications of AS<sup>1</sup>. However, there are numerous other potential applications for AS, including food research<sup>11</sup>. To replicate the properties of natural saliva, researchers have employed various chemical compounds that are applicable to a wide range of applications. For instance, carboxymethylcellulose (CMC) simulates the mucin<sup>12</sup>.

The objective of this investigation is to identify the AS composition that is selected most frequently in each of the preceding research categories. We will also determine whether a correlation exists between different compositions and whether their use is evenly distributed within each study category. AS is a medium used to evaluate how materials react to oral cavity<sup>13</sup>, alternatively, it can function as a control to evaluate the impact of other ingested solutions on a material that going to be used in oral cavity<sup>9,14</sup>. The contribution of this study is to provide researchers with a guide for selecting an AS type for their experiment. It also aims to promote the standardization of an AS combination within the research community, which would facilitate the replication of experiments.

One of this study's limitations is that we do not experimentally compare the existing solutions; therefore, it is uncertain whether they are ultimately effective. Additional *in vitro* trials are required to verify the accuracy and impartiality of the data found in the literature.

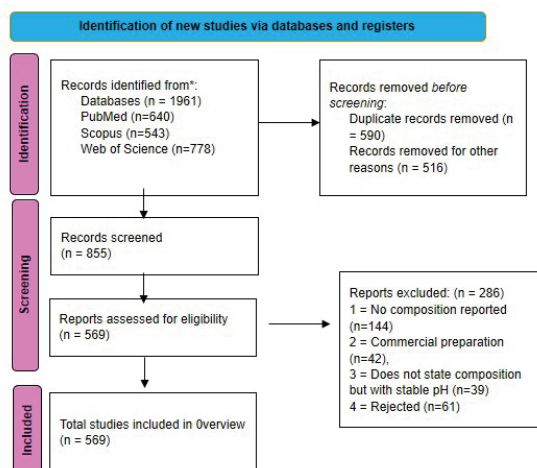
## Methodology

The study type is Literature Research: An Up-to-Date Overview. The implemented methodology aims to identify common AS solutions and provide guidance to the community regarding their diverse applications and combinations.

**Data sources:** Data Sources and Search Strategy PubMed, Scopus, and Web of Science databases were used for the present search, encompassing articles from the preceding decade. The keywords used were “artificial saliva”, “*in vitro* studies”, and “AS”. Inclusion criteria stipulated articles that reported results using AS in a laboratory (*in vitro*) setting, without restrictions on the

country of origin. Exclusion criteria disqualified articles that comprised experiments on laboratory animals or humans (*in vivo* studies), experiments utilizing natural saliva, or articles for which the full text could not be obtained. Additionally, abstracts, conference proceedings, author editorials, and book chapters were excluded. Articles were also excluded if they did not present reliably extracted data or were found to be duplicates.

**Data Extraction and Analysis** The initial search yielded 1961 articles (PubMed: 640; Scopus: 543; Web of Science: 778). After automated duplicate removal, 1371 articles remained. During this phase, any remaining duplicates that had not been identified by the program were manually removed. Studies were then categorized (*normalized*) by study type. Information on the AS composition was recorded for each study. After screening the abstracts for categorization, the total number of articles was reduced to 855. Following a full-text analysis based on the exclusion criteria, a further 286 articles were excluded. The final selection amounts to 569 articles.



Finally, the main categories introduced herein were labeled according to the types of materials added to them. Labels were designated as follows with 0 or 1. S when salts were present, U when urea was added, E when enzymes were added, P when proteins were added, E0 when preservatives had been placed, and at the end, N when nutrients had been added to the solution.

## Statistical Methodology

The sample of data under analysis constituted 569 valid cases from a total of 855 publications, spanning the years 2012–2022. For the 286 (out of 855) publications that were not included, a case-by-case justification was given. Papers that were rejected either did not mention a formulation; it was a commercial formulation with an unknown composition; they did not mention the formulation (mentioning only the pH of the solution); or they were rejected based on the exclusion criteria.

A total of 25 distinct compositions were identified within the valid publications, of which only a small number stood out in terms of their frequency of use. Based on the literature, the publications were divided into five groups. The frequency of occurrence of compositions in each group and during the respective years was then studied.

The hypothesis that the compositions appear with the same frequency during the 11 years was tested using the Generalized Estimating Equations (GEE) model. In this model, the frequency of occurrence of the compositions per time was defined as the dependent variable, assuming an appropriate Poisson distribution. Pairwise comparisons were performed, considering the correction of Type I Error with the Bonferroni method. This procedure was followed for each of the 5 bibliographic groups. The statistical analysis was performed using the R programming language<sup>15</sup> and the libraries gee<sup>16</sup> and emmeans<sup>17</sup> while in each statistical test the level of statistical significance was set for  $p < 0.05$ .

**Study methodology.** In each group, valid publications (with no missing ingredients) between 2012 and 2022 (an 11-year period) were found. Comparison of occurrence frequencies was performed using Generalized Estimating Equations and the assumption that the occurrence distribution of the solutions is Poisson. Comparisons between solutions were made by pairwise comparisons of estimated mean occurrence rates, with p-value correction using the Bonferroni method. The comparison did not include solutions that appeared less than 3 times. The final subgroups are set based on the frequency with which they appear in each research group.

### 3. Results

3.1.1 Data Check – Lost Values. The data file contains N = 855 article. The column ‘Missing’ contains N1 = 286 article of justification for missing values with categories: 1 = Does not state composition, 2 = Commercial formulation, 3 = Does not state composition but with constant pH, and 4 = Rejected. Therefore, the remaining cases are N = 569.

3.1.2 Search for the different compositions in N = 569 valid papers. In this subsection the corresponding statistical results were summarized in Table 1.

Table 1. Compositions in the literature and their frequency of occurrence

Composition Code	S	U	E	P	E0	N	Frequency (N)	%
33	1	0	0	0	0	0	239	42
49	1	1	0	0	0	0	115	20.2
37	1	0	0	1	0	0	51	9
39	1	0	0	1	1	0	24	4.2
34	1	0	0	0	0	1	21	3.7
54	1	1	0	1	0	1	18	3.2
50	1	1	0	0	0	1	16	2.8
38	1	0	0	1	0	1	14	2.5
45	1	0	1	1	0	0	10	1.8
35	1	0	0	0	1	0	9	1.6
61	1	1	1	1	0	0	9	1.6
41	1	0	1	0	0	0	8	1.4
62	1	1	1	1	0	1	7	1.2
40	1	0	0	1	1	1	5	0.9
46	1	0	1	1	0	1	5	0.9
57	1	1	1	0	0	0	4	0.7
36	1	0	0	0	1	1	3	0.5
53	1	1	0	1	0	0	3	0.5
52	1	1	0	0	1	1	2	0.4
5	0	0	0	1	0	0	1	0.2
6	0	0	0	1	0	1	1	0.2
42	1	0	1	0	0	1	1	0.2
44	1	0	1	0	1	1	1	0.2
56	1	1	0	1	1	1	1	0.2
58	1	1	1	0	0	1	1	0.2

Table 1 footnote: The first column indicates the composition codes that will be used in the text. In the total of 569 publications, the first three compositions in terms of frequency of their selection by researchers are: “33” with a frequency of 239 (42%), which corresponds to a composition containing only S, “49” with a frequency of 115 (20.2%), which corresponds to a composition with S and U, and “37” with a frequency of 51 (9%), which corresponds to a composition with S and P.

### 3.2 The use of compositions in groups based on literature

3.2.1 The group: ‘Biofilm studies, microbiological study and interaction of substances’. In this group N=54 valid publications were found. Out of 25 total compositions, only 12 have been used in the first group, (group 1) of literature. In Table 2, the subgroups formed within group 1 were summarized according to the frequency of use of the compositions.

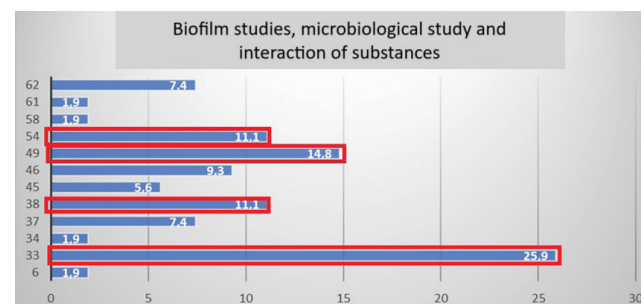


Figure 1. Breakdown of the use of Solutions in group 1 aggregated

Solution 33(S) is the sole solution with a rate of occurrence per year that exceeds unity and exhibits a statistically significant increase in frequency compared to any other solution, ranging from 1.7 to 4.7 times,  $p < 0.001$ . In second place is partially 49(S,U), with an occurrence rate per year of  $0.73 < 1$  it and appears statistically significantly more times than 37(S,P), 62(S,U,E,P,N), and 45(S,E,P), while finally 45(S,E,P) appears statistically significantly fewer times than 38(S,P,N) and 54(S,U,P,N). Hence, the compositions discovered to be employed by

the initial group of the literature are classified into the fourth subgroup based on their frequency of utilization. Of course, apart from subgroup A, which is characterized by utilizing composition 33(S), subgroups B and C use common compositions 38(S,P,N), 54(S,U,P,N), and 46(S,E,P,N), and subgroups C and D use common compositions 46(S,E,P,N), 37(S,P) and 62(S,U,E,P,N) (Figure 1). The subgroups of the first group of literature are presented in Table 2.

Table 2. Subgroups in terms of frequency of use of compositions.

A. Most frequent	B.Regular frequent	C.Low frequent	D.Rare frequent
<b>33 (N=14, 25.9%)</b>	<b>49 (N=8, 14.8%)</b>	38 (N=6, 11.1%)	46 (N=5, 9.3%)
	38 (N=6, 11.1%)	54 (N=6, 11.1%)	37 (N=4, 7.4%)
	54 (N=6, 11.1%)	46 (N=5, 9.3%)	62 (N=4, 7.4%)
	46 (N=5, 9.3%)	37 (N=4, 7.4%)	<b>45 (N=3, 5.6%)</b>
		62 (N=4, 7.4%)	

Table 2 footnote: Number N is enclosed within parentheses, followed by the percentage of the total of 54 publications of the group 'Biofilm studies, microbiological study, and interaction of substances'. Compositions appearing in exactly one subgroup are highlighted in bold.

3.2.2 The group: 'Studies of changes in chemical properties of materials'. A total of 163 valid publications were identified within this group. Out of a total of 25 compositions, only 17 have been employed in the second group of literature. Table 3 shows the subgroups formed within group 2 according to the frequency of use of the compositions and according to the results of the pairwise comparisons between the compositions. It is important to note that only the next seven compositions appear with a frequency equal to or greater than 3: 33(S), 34(S,N), 36(S,E0,N), 37(S,P), 45(S,E,P), 49(S,U), and 54(S,U,P,N). In fact, 76.1% of the publications mention either composition 33(S) or 49(S,U). The analysis will be performed based on these seven compositions.

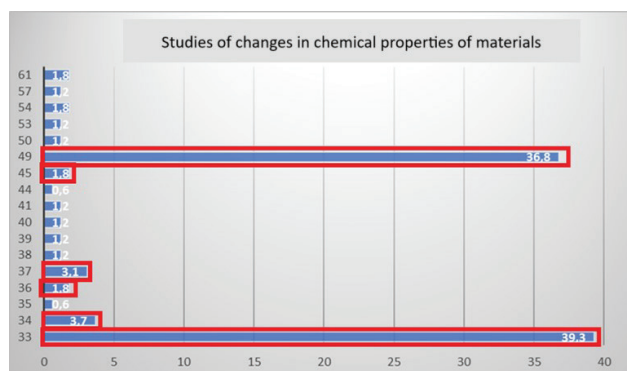


Figure 2. Breakdown of Solutions uses in group 2 aggregated

The conclusion is that solutions 33(S) and 49(S,U), with an occurrence ratio of 39.3% and 36.8%, are the only ones with an occurrence rate per year greater than unity (6.4 and 6.0 respectively) and appear statistically

significantly more frequently than the rest (specifically from 10.7 to 21.3 times more,  $p < 0.001$ ). In second place are 34(S,N) and 37(S,P), with an occurrence rate per year of 0.6 and  $0.5 < 1$ , respectively (Figure 2). But composition 37(S,P) is also categorized in the third subgroup. Therefore, the compositions found to be used by the second group of the literature are divided into three subgroups based on their frequency of use. The subgroups of the 2<sup>nd</sup> literature group are presented in Table 3.

Table 3. Subgroups in terms of frequency of use of compositions.

A. Most frequent	B.Regular frequent	C.Low frequent
<b>33 (N=64, 39.3%)</b>	<b>34 (N=6, 3.7%)</b>	37 (N=5, 3.1%)
<b>49 (N=60, 36.8%)</b>	37 (N=5, 3.1%)	<b>36 (N=3, 1.8%)</b>
		<b>45 (N=3, 1.8%)</b>
		<b>54 (N=3, 1.8%)</b>
		<b>61 (N=3, 1.8%)</b>

Table 3 footnote: Numeral N is enclosed within parentheses, followed by the percentage of the total of 163 publications of the group 'Studies of changes in chemical properties of materials'. Compositions appearing within exactly one subgroup are marked in bold.

3.2.3 The group: 'Studies of micromechanical properties of surface materials'. In this group, there were 85 valid publications. Only 12 of the 25 compositions have been used in the third group of literature. It is noteworthy that out of the 12 combinations, only four appear with a frequency equal to or greater than 3: 33(S), 34(S,N), 37(S,P) and 49(S,U). Therefore, the analysis will be based on these four components. Table 4 shows the subgroups formed within group 3 according to the frequency of use of the compositions and according to the results of the pairwise comparisons between the compositions.

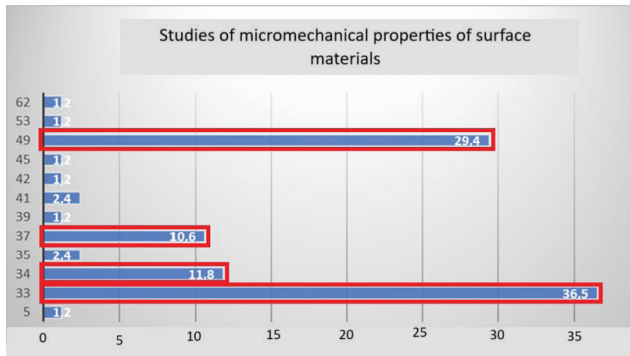


Figure 3. Breakdown of Solutions is used in group 3 aggregated.

In group 3, the four compositions that appear most often (the remaining 8 - out of 12 - that were not included in the statistical model have appeared from 1 to 2 times) are 33(S) (N=31, 36.5%), 49 (N=25, 29.4%), 34 (N=10, 11.8%) and 37 (N=9, 10.6%) and their average occurrence rate surpasses 1, so unlike groups 1 and 2 more choices are observed compositions (Figure 3). According to previous results, the compositions found to be used by the third group of literature in at least three publications are divided into three subgroups according to their frequency of use. The third literature group is presented as subgroups in Table 4.

Table 4. Subgroups are defined by their frequency of use of compositions.

A. Most frequent	B. Regular frequent	C. Low frequent
33 (N=31, 36.5%)	49 (N=25, 29.4%)	34 (N=10, 11.8%) 37 (N=9, 10.6%)

Table 4 footnote: N is followed by the percentage of the total 85 publications of the group ‘Studies of micromechanical properties of surface materials.’.

Table 5. Subgroups are distinguished by their frequency of utilization of compositions.

A. Most frequent	B. Most frequent	C. Regular frequent	D. Regular frequent	E. Low frequent	Z. Rare frequent
33 (N=118, 49%)	37 (N=33, 13.8%)	39 (N=20, 8.4%) 49 (N=17, 7.1%)	49 (N=17, 7.1%) 50 (N=14, 5.9%)	54 (N=9, 3.8%)	35 (N=6, 2.5%) 38 (N=5, 2.1%) 34 (N=4, 1.7%) 40 (N=3, 1.3%) 41 (N=3, 1.3%)

Table 5 footnote: In parentheses is number N, followed by a total of 240 publications of the group ‘Studies of dental tissue behavior / biocompatibility.’.

3.2.5 The group: ‘Food-Drug Studies’. A total of 27 valid publications were identified within this group. Only eight of the 25 total compositions have been used in the fifth group of literature. It is significant that out of the 8 combinations, only the following four appear with a frequency equal to or greater than 3: 33(S), 49(S,U), 45(S,E,P), and 61(S,U,E,P). Therefore, the analysis will

3.2.4 The group: ‘Behavioral studies of dental tissues / biocompatibility’. This group had N=240 valid publications. Out of the total of 25 compositions, 16 have been employed in the fourth group of literature. The following five combinations appear with a frequency equal to or less than two: 52(S,U,E0,N), 56(S,U,P,E0,N), 57(S,U,E), 61(S,U,E,P), and 62(S,U,E,P,N). These five compositions will be removed from the statistical analysis model, which will be based on the remaining 11 compositions. It is interesting to note that compound 33(S) appears in 49% (S,U) of the publications. Table 5 illustrates the subgroups formed within group 4 according to the frequency of use of the compositions and the results of the pairwise comparisons between the compositions.

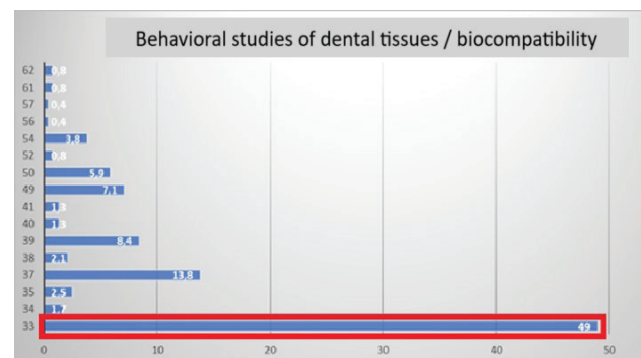


Figure 4. Breakdown of Solutions uses in group 4 aggregated.

In group 4, 7 subgroups were found as presented in Table 4. The most important in terms of composition frequency are: subgroup A, use of composition 33(S), N=118, 49%, subgroup B, use of composition 37(S,P), N=33, 13.8%, subgroup B characterized by composition 39(S,P,E0), N=20, 8.4% and 49(S,U), N=17, 7.1%, while 49(S,U) is also present in subgroup D with 50, N=14, 5.9%, while then the compositions in groups E and Z (Figure 4) (Table 5) have a much lower frequency of occurrence.

be conducted utilizing these four compositions. Figure 5 and Table 6 provide the incidence for each year and the overall rate. Table 6 depicts the subgroups derived from group 5 based on the frequency of utilization of the compositions and the outcomes of pairwise comparisons between the compositions.

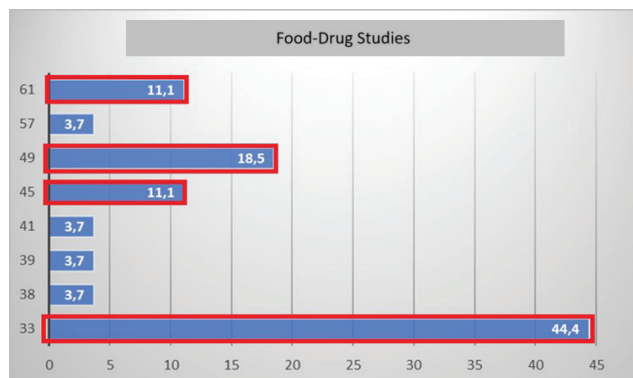


Figure 5. Breakdown of Solutions is used in group 5 aggregated.

In group 5, four compositions appear at least three times. They are 33(S) (N=12, 44.4%), 49(S,U) (N=5, 18.5%), 45(S,E,P) (N=3, 11.1%) and 61(S,U,E,P) (N=3, 11.1%). According to previous results, the compositions found to be used by the fifth group in the literature are divided into two subgroups based on their frequency of use (Figure 5). These are the subgroups A, composed of 33, and B, comprising 49, 45(S,E,P), and 61(S,U,E,P). The subgroups of the fifth literature group are depicted in Table 6.

Table 6. Subgroups are distinguished by their frequency of utilization of compositions.

A. Most frequent	B. Regular frequent
33 (N=12, 44.4%)	49 (N=5, 18.5%)
	45 (N=3, 11.1%)
	61 (N=3, 11.1%)

Table 6 footnote: In parentheses is the number N and then the percentage of the 23 (out of a total of 27) publications of the 'Food Studies - Medicines' group.

By observing the groups, the subsequent inferences may be drawn: The Food-Drug Studies group demonstrated the smallest possible selection in the composition of AS by creating only two subgroups statistically. Compared to all other groups, we have the most choices in studies of dental tissue behavior/biocompatibility. Six subgroups of comparisons arise. 'Studies of biofilm, microbiological study, and interaction of substances' shows four subgroups, and the other two groups show three subgroups with fewer options for the frequency of use of AS compositions. There may be many choices in composition, or the groups may have shown a large amount of study.

### 3.3 The use of compositions in AS groups based on literature.

There were 276 publications found with the keyword 'Inorganic + Organic solution.' Except for compositions 5 and 6, each other one was found in at least one publication. The compositions of 49 with 73 publications

(26.4%), 37 with 41 (14.9%) publications, and 33 with 28 (10.1%) publications were identified with a percentage greater than 10%.

- i) 99 publications were identified within the "inorganic solution only" keyword, and out of these, 91 (91.9%) utilized 33, 6 (6.1%) 49, and 2 (2%) 34.
- ii) 60 publications were found with the "Hepes solution" keyword, and of these, 55 (91.7%) used 33, 2 (3.3%) 41, 2 (3.3%) 54 and one (1.7%) 45.
- iii) Of the 53 publications found in the keyword 'TRIS solution,' 40 (75.5%) used 33, 10 (18.9%) 37, and one (1.9%) each used 39, 41, and 49.
- iv) Of the 40 publications found with the "Fusagyama-M" keyword, 49 were used in 29 (72.5%), 33 in 9 (22.5%), and 53 in the remaining two (5%).
- v) Ten publications were identified within the "SAGF" keyword, out of which six (60%) utilized 33, whereas the remaining four (40%) utilized 49.
- vi) Six publications found the 'Klimek' keyword, out of which five (83.3%) used 54 and one (16.7%) used 50.
- vii) Five publications were identified within the "CAS" keyword, and among them, four (80%) utilized 62, whereas one (20%) utilized 61.
- viii) Two publications were found with the keyword 'SOF' with composition 33.
- ix) Finally, in the "NA" keyword, 16 publications were found, 4 (43.8%) with 33, 2 (12.5%) with 34 and 38 respectively, and finally from one (6.2%) 5, 6, 35, 45, and 49.

## Discussion

Researchers highlight the necessity of a guide to facilitate the appropriate selection of AS. Diverse compositions are available in the literature for the same research objective. The objective of the current investigation is to assist researchers in understanding the properties of the solutions and to facilitate their decision-making regarding the composition. In the long term, the objective of this investigation is to contribute to the enhancement of solutions for future research. The primary limitation of this presentation is its inability to compare the various types of AS, as they require in vitro testing.

The most important property of AS is its function as a buffer solution to maintain a constant pH. Almost all studies utilize organic or inorganic salts in AS to maintain a constant pH. Thus, they can observe the evolution of a material at a constant pH or by changing it using thermal cycling<sup>18</sup>. Consequently, salt is the initial member of each category. Among the best-known organic chemicals used are HEPES buffer<sup>19</sup> or Tris buffer<sup>20</sup>. HEPES buffer, which also belongs to the Good's buffer group<sup>21</sup>. During its application in simulated body fluid solutions (SBF,

an inorganic solution with a similar composition to human blood plasma without any organic components), it demonstrated significant activity in the corrosion of glass-ceramics, leading to the recommendation of utilizing another buffer<sup>21</sup>. Several studies have shown that compositions with only inorganic formulations have different concentrations of minerals in the solution. Compositions that are quite common include Klimek<sup>22-24</sup> or the Fusayama–Meyer formula with urea<sup>6,25,26</sup> and the SAGF-medium<sup>27,28</sup>.

Another common compound added to the aqueous solution is urea. As a chemical, urea is not one of the primary components of natural saliva. Urea, also known as carbamide, is an organic compound with a molecular weight of 60 Da<sup>29</sup>. It appears in the oral cavity in the case of uremic stomatitis<sup>30</sup>. The utilization of urea in AS assists in maintaining the pH level of the solution and regulating the presence of microorganisms within the solution<sup>29</sup>. As evidenced by this investigation, it is the second most common option for researchers.

Proteins are another material used in AS. In natural saliva, glycoproteins help modify the viscosity and solubility of substances in the solution<sup>2</sup>. The most commonly occurring protein is mucin<sup>2</sup>. Mucin plays a significant role in chewing food and enhances the sense of touch within the oral cavity. In several studies, alternative formulations have been employed, including Xanthan gum as a coagulant<sup>31</sup> or Propylene Glycol<sup>32</sup> or carboxymethylcellulose CMC<sup>12</sup>, which alter the viscosity of the AS. In some articles, adding mucin to AS was helping to accelerate Pellicle formation, a complex process that involves the development of a thin, non-mineralized layer of salivary proteins on the enamel surface of teeth<sup>33</sup>. As evidenced by the data, it has been implemented in limited quantities in all groups except the food study group, providing researchers with a more accurate representation of oral cavity conditions.

Another important group of proteins are enzymes. Enzymes catalyze intense chemical reactions and contribute to the hydrolysis of peptides. Researchers may accelerate chemical reactions, such as those that occur in the oral cavity, with the assistance of enzymes. This is the reason for this group's extensive diversity and its relative rarity in AS formulations. The most commonly used enzymes are Lysozyme, Amylase, and Alkaline phosphatase<sup>34</sup>. The concentrations used are amylase from 200-500 KU/L<sup>13,35</sup> and lysozyme at a concentration of 750 U/L<sup>36</sup>. The composition "Complete Artificial Saliva" (CAS) also includes alkaline phosphatase at a concentration of 4 U/L<sup>34</sup>. The use of proteolytic enzymes in AS can biodegrade natural collagen types of sutures that are used in oral surgery<sup>13</sup>.

Subsequently, another factor that alters the composition is the presence of microbes. In several studies, it was necessary to culture microorganisms, and nutrients were used. The best known is glucose<sup>37</sup>. To

obtain the microbiome of the oral cavity, researchers mix both natural and AS<sup>38</sup>. In other research groups, whose protocols prohibited microorganism growth, the utilization of preservative formulations was employed to inhibit colony growth without affecting the research process. Examples of formulations include carboxymethylchitosan or sodium azide<sup>39</sup> or methyl 4-hydroxybenzoate<sup>12</sup>. Some other studies used antibiotics such as 100 U/ml penicillin and 1,000 µg/ml streptomycin<sup>40,41</sup> in the formulation of AS to control microbial growth on the specimens.

Vitamin C is another used component of AS solutions<sup>23,42,43</sup> and is also a commonly used positive control for the free radical scavenging antioxidant activity test. It has been demonstrated that vitamin C inhibits the cytotoxic and apoptotic effects of *P. gingivalis* in human gingival fibroblasts<sup>44</sup>.

In a substantial proportion of studies, no reference was made to the composition of AS<sup>45</sup>. In a percentage of these, the pH is mentioned without referring to anything else about the composition<sup>46</sup>. These studies were deemed missing values and were examined separately during the statistical analysis. Ultimately, commercially available compositions exist that can be procured to carry out the experiment, such as the commercial formulations Aqwet<sup>47</sup>, Wet mouth<sup>48</sup>, Caphosol<sup>®49</sup>, Farmácia Santa Paula – Araraquara, Saliform<sup>50</sup>, Biotène Oral<sup>51</sup> and Glandosane<sup>®52</sup>. In these studies, there is no reference to the composition of the commercial formulation; instead, only the name of the commercial formulation is mentioned.

Casein could be used as a potential salivary phosphoprotein homolog for in vitro dentin remineralization studies<sup>53</sup>. According to the study of Nakamura et al., 2021<sup>54</sup>, casein is one of the most abundant proteins in milk, and its biological function is primarily nutritional. Casein has been shown to inhibit erosion and decalcification in oral health.

Several studies have described the storage of AS. It is recommended that the preparation be stored in a dark location until the execution of the experiment<sup>9</sup>. The preservation temperature has been specified as -20°C<sup>9,55</sup>

## Conclusion

Studies employing AS in vitro address a diverse array of investigative objectives and inquiries. This is necessary given that the properties of AS are far from mimicking those of natural saliva. Specific AS formulas were needed for specific studies that could be used in a research category. In addition, numerous studies do not disclose the composition or preparation method of AS. The most frequently occurring AS formula in the current literature review was composed entirely of salts, while the second most frequently occurring formula was a combination

of salts and urea. Additional research is required to determine the optimal AS for each research field, which will serve as a standard for future researchers and generate more reliable results in future research.

### Limitation of this study

We are unable to ascertain the effectiveness of the existing solutions because the researchers have not conducted a comparison. To ensure that the data in the literature is impartial and accurate, it is necessary to conduct additional in vitro trials. Additionally, the present study is constrained by the relatively high proportion of studies that lack sufficient information regarding the formula of AS. Another potential limitation is derived from our methodology: we categorize studies in a manner that is inherently arbitrary, which means that homogeneity within groups cannot be guaranteed.

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