Indian Journal of Research in Homoeopathy

Volume 18 | Issue 3 Article 3

27-9-2024

Stability study of homoeopathically potentised Arsenicum album in hydrolytic amber glass class II and high-density polyethylene terephthalate over twelve months

Marcia Pietroluongo
Laboratory of Experimental Pharmacotechnics, Department of Galenic Innovation, Vice-Directorate of Education, Research and Innovation, Institute of Technology in Pharmaceuticals - Farmanguinhos, Oswaldo Cruz Foundation - FIOCRUZ, Rio de Janeiro, Brazil, marcia.pietroluongo@fiocruz.br

Thiago Frances Giuimarães
Laboratory of Experimental Pharmacotechnics, Department of Galenic Innovation, Vice-Directorate of Education, Research and Innovation, Institute of Technology in Pharmaceuticals - Farmanguinhos, Oswaldo Cruz Foundation - FIOCRUZ, Rio de Janeiro, Brazil, thiago.frances@fiocruz.br

Thais de Barros Fernandes
Multidisciplinary Laboratory of Pharmaceutical Sciences, Department of Drugs and Medications, Pharmacy College, Federal University of Rio de Janeiro - UFRJ, Rio de Janeiro, Brazil, fernandes.thaisb@gmail.com

Ana Paula dos Santos Matos
Laboratory of Experimental Pharmacotechnics, Department of Galenic Innovation, Vice-Directorate of Education, Research and Innovation, Institute of Technology in Pharmaceuticals - Farmanguinhos, Oswaldo Cruz Foundation - FIOCRUZ, Rio de Janeiro, Brazil, paula.matos@fiocruz.br

Fortune Homsani
Multidisciplinary Laboratory of Pharmaceutical Sciences, Department of Drugs and Medications, Pharmacy College, Federal University of Rio de Janeiro - UFRJ, Rio de Janeiro, Brazil, fortune.homsani@gmail.com

See next page for additional authors

Author(s) ORCID Identifier:
https://orcid.org/0000-0002-3944-6486
https://orcid.org/0000-0002-4534-6414
https://orcid.org/0000-0002-2691-5335
https://orcid.org/0000-0001-8511-0003
https://orcid.org/0000-0002-6441-7281
https://orcid.org/0000-0003-2003-579X
https://orcid.org/0000-0001-6968-4269
https://orcid.org/0000-0001-5086-5676

How to cite this article

Pietroluongo M, Giuimarães TF, de Barros Fernandes T, Dos Santos Matos AP, Homsani F, Oliveira AP, et al. Stability study of homoeopathically potentised Arsenicum album in hydrolytic amber glass class II and high-density polyethylene terephthalate over twelve months. Indian J Res Homoeopathy 2024;18:172-179.

Stability study of homoeopathically potentised Arsenicum album in hydrolytic amber glass class II and high-density polyethylene terephthalate over twelve months

Abstract

Background: The Brazilian Homoeopathic Pharmacopoeia (BHP) recommends using glass amber Type I, II, or III as a primary container for storing homoeopathic liquid medicines. Although the glass has advantages, there are some disadvantages, such as fragility, weight, and the presence of silicate particles, which have been described as critical components involved with the generation of silicon microparticles detected in homoeopathic solutions. **Objectives:** The present study aimed to investigate the stability of hydroalcoholic solutions of *Arsenicum album* dynamised in two different vials: class II hydrolytic amber glass and amber high-density polyethylene terephthalate (PET). **Materials and Methods:** Two potencies of the *Arsenicum album*, 6C and 30C, were prepared according to BHP. The solutions were stored in glass and PET amber flasks. The stability study was carried out in a climatic chamber according to the conditions described in the Brazilian official compendium for twelve months. During the study, samples were evaluated by density, pH, refractometry, and conductivity. Microbiological purity was evaluated at the beginning and the end of stability study. **Results:** No statistical differences were observed in the density of samples in both conditions and packages. The pH of all samples remained around 6, and refractometry around 1.363, suggesting their chemical stability. The microbiological assays showed no pathogenic microorganisms after twelve months of storing all samples. **Conclusion:** The results showed that *Arsenicum album* 6C and 30C are stable preparations. It can also be concluded that glass bottles are suitable for storing dynamised solutions of *Arsenicum album*. PET bottles maintained the physicochemical and microbiological stability of the solutions and can be a potential alternative to prepare or store homoeopathic matrices. However, further studies are required to confirm the suitability of PET vials in compounding and storing homoeopathic medicines.

Acknowledgments and Source of Funding

Source Funding We thank the Brazilian Association of Homeopathic Pharmacists (Associação Brasileira de Farmacêuticos Homeopatas—ABFH) for partially funding this study. Acknowledgments The authors would like to thank the Quality Control Laboratory of Farmanguinhos, Fiocruz, for making the use of climatic chambers available. In addition to Dra. Helena Keiko Toma for microbiologic analysis support.

Authors

Marcia Pietroluongo, Thiago Frances Giuimarães, Thais de Barros Fernandes, Ana Paula dos Santos Matos, Fortune Homsani, Adriana Passos Oliveira, Alessandra Lifsitch Viçosa, and Carla Holandino Quaresma

Original Article

Stability study of homoeopathically potentised *Arsenicum album* in hydrolytic amber glass class II and high-density polyethylene terephthalate over twelve months

Marcia Pietroluongo[1], Thiago Frances Giuimarães[1], Thais de Barros Fernandes[2], Ana Paula dos Santos Matos[1], Fortune Homsani[2], Adriana Passos Oliveira[2], Alessandra Lifsitch Viçosa[1], Carla Holandino Quaresma[2]*

[1]Laboratory of Experimental Pharmacotechnics, Department of Galenic Innovation, Vice-Directorate of Education, Research and Innovation, Institute of Technology in Pharmaceuticals - Farmanguinhos, Oswaldo Cruz Foundation - FIOCRUZ, Rio de Janeiro, Brazil, [2]Multidisciplinary Laboratory of Pharmaceutical Sciences, Department of Drugs and Medications, Pharmacy College, Federal University of Rio de Janeiro - UFRJ, Rio de Janeiro, Brazil

Abstract

Background: The Brazilian Homoeopathic Pharmacopoeia (BHP) recommends using glass amber Type I, II, or III as a primary container for storing homoeopathic liquid medicines. Although the glass has advantages, there are some disadvantages, such as fragility, weight, and the presence of silicate particles, which have been described as critical components involved with the generation of silicon microparticles detected in homoeopathic solutions. **Objectives:** The present study aimed to investigate the stability of hydroalcoholic solutions of *Arsenicum album* dynamised in two different vials: class II hydrolytic amber glass and amber high-density polyethylene terephthalate (PET). **Materials and Methods:** Two potencies of the *Arsenicum album*, 6C and 30C, were prepared according to BHP. The solutions were stored in glass and PET amber flasks. The stability study was carried out in a climatic chamber according to the conditions described in the Brazilian official compendium for twelve months. During the study, samples were evaluated by density, pH, refractometry, and conductivity. Microbiological purity was evaluated at the beginning and the end of stability study. **Results:** No statistical differences were observed in the density of samples in both conditions and packages. The pH of all samples remained around 6, and refractometry around 1.363, suggesting their chemical stability. The microbiological assays showed no pathogenic microorganisms after twelve months of storing all samples. **Conclusion:** The results showed that *Arsenicum album* 6C and 30C are stable preparations. It can also be concluded that glass bottles are suitable for storing dynamised solutions of *Arsenicum album*. PET bottles maintained the physicochemical and microbiological stability of the solutions and can be a potential alternative to prepare or store homoeopathic matrices. However, further studies are required to confirm the suitability of PET vials in compounding and storing homoeopathic medicines.

Keywords: Amber glass flasks, *Arsenicum album*, Polyethylene terephthalate primary container, Stability study

Introduction

Homoeopathy is one of the most widespread forms of integrative medicine whose therapeutic efficacy is established by the experience of clinical use and by the research described in scientific literature. This therapy consists of four principles: The law of similia (*similia similibus curentur*), medicines experimentation in healthy individuals, minimum doses, and single medication.[1,2] Homoeopathic medicines are prepared by vigorous agitation and serial dilutions that potentiate the product and make it suitable for administration.[1]

The stability of pharmaceutical products depends on extrinsic factors (temperature, humidity, light) and intrinsic to the product, such as physicochemical properties, manufacturing process, properties, and type of packaging materials. The study of the impact generated by these factors can predict the pharmaceutical product's shelf life, period of use in

Access this article online	
Quick Response Code: Available in print version only	Website: www.ijrh.org
	DOI: 10.53945/2320-7094.2003

***Address for correspondence:** Carla Holandino Quaresma, UFRJ, Rio de Janeiro, Brazil.
E-mail: cholandino@gmail.com

Received: 07 December 2023; **Accepted:** 02 September 2024

How to cite this article: Pietroluongo M, Giuimarães TF, de Barros Fernandes T, Dos Santos Matos AP, Homsani F, Oliveira AP, *et al*. Stability study of homoeopathically potentised *Arsenicum album* in hydrolytic amber glass class II and high-density polyethylene terephthalate over twelve months. Indian J Res Homoeopathy 2024;18:172-179.

packaging, and specified storage conditions.[3] The stability of homoeopathic mother tinctures matrices is a subject of great interest for pharmacies and laboratories that manufacture, manipulate and produce homoeopathic medicines. The Brazilian Homoeopathic Pharmacopoeia (BHP) recommends using amber coloured glass Type I, II, or III as the primary storage container for liquid medicines.[4] The fragility, weight, and generation of silicon microparticles detected in homoeopathic solutions are disadvantages attributed to glass storage. Therefore, studies using different packaging materials can provide support for future research.

The present study was conducted with *Arsenicum album* (arsenic trioxide), one of the oldest remedies that has it's pathogenesis extensively described in various homoeopathic Materia Medica. This homoeopathic medicine relieves anxiety, urinary infection, stomach pain, skin irritation, and many others. Furthermore, high dilutions of *Arsenicum album* decrease the oxidative stress level. Dilutions 6C and 30C, used in this work, are the most used in the clinic.[5]

Hence, this study aimed to investigate the stability of hydroalcoholic solutions of *Arsenicum album* in hydrolytic amber glass Class II and high-density polyethylene terephthalate (PET) over twelve months according to the conditions and methods described in a normative ruling from the Brazilian Health Regulatory Agency,[6] to contribute with the quality control of magistralis homoeopathic preparations.

Materials and Methods

Arsenicum album potencies preparation

The *Arsenicum album* 3C was used as a matrix for the following dynamisations, according to BHP guidelines and published in a previous work.[4,7] Centesimal dilutions of *Arsenicum album* 6C and 30C were prepared according to the Brazilian Homeopathic Pharmacopoeia method for insoluble drugs. From the potency of the crushed *Arsenicum album* 3C, solubilisation was performed to obtain *Arsenicum album* 4C using water and 96% (w/w) alcohol. From the 4C potency, dilutions were made with 70% (w/w) alcohol. Vertical agitation promoted by a mechanical arm used for this purpose was used (Denise, AUTIC, São Paulo, Brazil). Samples were prepared in triplicate in Class II hydrolytic amber glass or PET (R18/400, Apace, São Paulo, Brazil) vials. In addition, vehicles (hydroalcoholic solutions 70% w/w) were also dynamised using the same method for both potencies (6C and 30C) as control during the stability study to compare results with samples. The samples were codified as: *Arsenicum album* 6C glass (*Ars. alb*.6C-G), *Arsenicum album* 6C PET (*Ars. alb*. 6C-P), *Arsenicum album* 30C glass (*Ars.alb*.30C-G), *Arsenicum album* 30C PET (*Ars.alb*.30C-P), Vehicle 6C glass (V6C-G), Vehicle 6C PET (V6C-P), Vehicle 30C glass (V30C-G), and Vehicle 30C PET (V30C-P).

Study of stability

Arsenicum album solutions were prepared and stored at both conditions: in a climatic chamber (model WK 13'/+20 – 45, Weiss Technik, USA) at 30°C ± 2°C and 75% ± 5% of relative humidity (RH), and room temperature and humidity (25°C ± 2°C and 60% ± 5% RH) for twelve months. Samples were taken at 0, 3, 6, 9, and 12 months and analysed according to visual aspects, density, pH, and refractometry. Samples of 0 and 12 months were also analysed by conductivity. Further, microbiological analyses were conducted at 0, 6 and 12 months (3). Table 1 presents the stability protocol used in this work. Table 2 shows the stability design.

Visual aspects and pH

All samples had their visual appearance analysed to identify changes in the appearance of the solution, such as color, clarity, or precipitate formation detectable macroscopically. About 7 ml of the sample was placed in a 10 ml test tube and observed on the aspects mentioned above. The pH value was measured in the same test tube, using Merck® universal pH indicator paper (0–14).

Determination of relative density

The samples' relative density was determined using a 10 ml glass pycnometer flask with an analytical balance.

The empty pycnometer weight was recorded (W_0) before it was filled with distilled water (W_w). The water temperature was recorded using the thermometer of the pycnometer. The internal volume of the pycnometer was determined, as shown by eq. 1.

$$V_0 = \frac{W_w - W_0}{\rho_w} \quad (1)$$

Where V_0 is the internal volume of the pycnometer, W_w is the weight of the pycnometer filled with distilled water, W_0 is the weight of the empty pycnometer and ρ_w is the water density

Table 1: Stability protocol for *Arsenicum album* and vehicles samples

Storage conditions	Storage time (months)				
	0	3	6	9	12
Room Temperature (25°C±2°C/60% RH±5%)	X	X	X	X	X
30°C±2°C/75% RH±5%	X	X	X	X	X

Legend: X – To be tested. RH: Relative humidity

Table 2: Stability design for *Arsenicum album* and vehicles samples

Test	Storage time (months)				
	0	3	6	9	12
Visual aspects	XY	XY	XY	XY	XY
Density	XY	XY	XY	XY	XY
pH	XY	XY	XY	XY	XY
Refractometry	XY	XY	XY	XY	XY
Conductivity	XY	-	XY	-	XY
Microbiological analyses	XY	-	XY	-	XY

Legend (storage condition): X- room temperature/Y–30°C±2°C/75±5%

at the temperature measured. The relative density of samples was determined according to eq. 2.

$$\rho_s = \frac{W_s - W_0}{V_0} \quad (2)$$

Where ρ_s is the relative density of the sample and W_s is the weight of the pycnometer filled with the respective sample. All determinations were made in triplicate.

Refractometry

Refractometry analyses were performed using an Abbe refractometer. The sample was placed in the prism and the device was adjusted using the lines of the light and dark fields until the refraction index was visualised on the scale.

Conductivity

For conductivity analyses, we used a Methron device. About 7 ml of the sample was placed in a test tube, where the equipment cell was introduced. The cell was washed with 70% w/w alcohol before each reading and the cell stabilisation time was 120 s.

Microbiologic assay

About 10 ml of the sample mixture was transferred to 90 ml of sodium chloride-peptone buffer solution (pH 7.0) to undertake the microbiological analyses. Successive decimal dilutions were prepared with the same diluent. The method used to identify the presence of microorganisms was plate counting using depth and surface methods. The microorganisms analysed were *Escherichia coli*, total microorganisms, fungi, and yeasts.[8]

Statistical analysis

The results were analyzed and compared by analysis of variance (ANOVA) and the Tukey test using the GraphPad Prism® 8.0 software (GraphPad Software, La Jolla, CA, USA), considering $p < 0.05$ as significant.

Results

Visual aspects and pH

No colour change, clarity, or precipitate formation was observed among the samples stored in the climatic chamber and at room temperature, with different packaging and potencies. All samples showed a pH range of 6.

Determination of relative density

The relative density of the samples taken in the pre-established time intervals of the stability study was determined and the results are presented in Table 3.

An ANOVA analysis was performed, and no statistically significant difference was found among the groups ($p > 0.05$).

Refractometry

The samples presented a refractive index of 1.363, which can be related to the pH values, suggesting homogeneous samples.

Conductivity

An analysis of the conductivity of the samples in both packages was also performed at the beginning and end of the stability study. The results are shown in Figure 1.

Initially, the dilutions of *Arsenicum album* 6C and 30C prepared and stored in amber glass bottles showed no statistically significant conductivity difference (2.370 ± 0.535 and 2.589 ± 0.119 µS.cm^{-1}, respectively) and not when compared to those stored in PET vials (2.626 ± 0.302 and 2.575 ± 0.125 µS.cm^{-1}) in the same dilutions in the time zero of storage. However, after 12 months of storage at room temperature, the solutions stored in amber glass flasks showed increased conductivity in both dilutions, 3.493 ± 0.047 µS.cm^{-1} solution of 6C and 3.499 ± 0.074 µS.cm^{-1} solution of 30C. Those stored in a climatic chamber showed even higher results, 4.366 ± 0.005 and 4.334 ± 0.025 µS.cm^{-1}, respectively.

On the other hand, the same dilutions prepared and stored in PET vials presented conductivity of 2.626 ± 0.302 and 2.575 ± 0.125 µS.cm^{-1}, that is, values very close to those initially stored in amber glass bottles. In this case, the conductivity of the solutions decreased after 12 months of storage. The 6C solution showed conductivity of 0.800 ± 0.067 µS.cm^{-1} (room temperature) and 0.924 ± 0.028 µS.cm^{-1} (climatic chamber) at the end of the stability study. In addition, 30C solutions showed conductivity of 0.806 ± 0.085 and 0.914 ± 0.070 µS.cm^{-1} under the respective conditions.

Using ANOVA one-way with Tukey as a post-test, it was verified that *Arsenicum album* samples, in both dilutions, prepared and stored 12 months in the two conditions (room temperature and climatic chamber) presented statistical

Table 3: Relative density of *Arsenicum album* potencies along the stability study prepared and stored in amber glass and PET flasks

Samples	Relative density (g/mL)				
	T_0	T_3	T_6	T_9	T_{12}
		Climatic chamber/room temperature			
Ars.alb 6C-G	0.860±0.0012	0.855±0.0004/0.855±0.0007	0.856±0.0015/0.855±0.0010	0.858±0.0012/0.857±0.0014	0.858±0.0014/0.859±0.0007
Ars.alb 30C-G	0.861±0.0009	0.858±0.0013/0.857±0.0008	0.858±0.0015/0.856±0.0003	0.856±0.0024/0.858±0.0006	0.858±0.0004/0.856±0.0006
Ars.alb 6C-P	0.861±0.0005	0.859±0.0022/0.857±0.0023	0.858±0.0016/0.857±0.0019	0.857±0.0019/0.858±0.0018	0.857±0.0001/0.858±0.0008
Ars.alb 30C-P	0.859±0.0007	0.858±0.0007/0.857±0.0002	0.858±0.0007/0.856±0.0008	0.856±0.0003/0.858±0.0014	0.857±0.0002/0.858±0.0004

*Both C and cH mean centesimal scale. PET: Polyethylene terephthalate

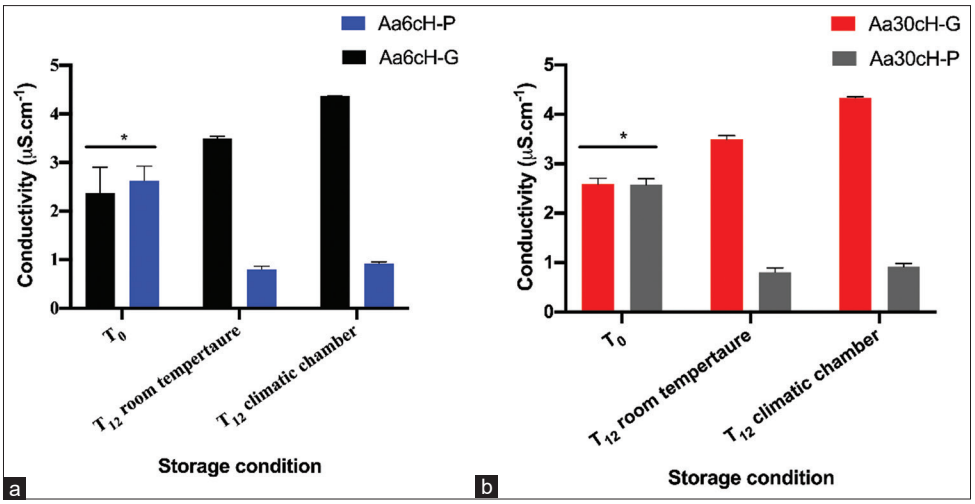

Figure 1: Conductivity results of *Arsenicum album* potencies in different storage conditions: (a) samples in dilution 6C and (b) samples in dilution 30C. (*) denotes no significant statistical difference ($p > 0.05$)

difference ($p < 0.05$) between samples stored in amber glass bottles and samples stored in PET.

Microbiologic assay

The microbiologic analyses indicated no microbial growth in all *Arsenicum album* preparations.

Discussion

The stability of homoeopathic medicines is a matter of great interest for pharmacies and laboratories that produce homoeopathic medicines. However, this topic still needs more technical-scientific investigations.

The *Arsenicum album* samples used in this study showed no change in color, visual aspect, pH, or refractometry. The results were already expected due to the mineral origin of the samples and the absence of active organic molecules to be degraded by hydrolysis or oxidation.

Conductivity measures the concentration of ions in the solution, proportional to the current flowing between the electrodes. For current to flow, ions must be present in the solution to carry the charge from one electrode to the other. When the number of ions in the solution increases, the amount of charge transported between the electrodes increases the conductivity.[9]

The physicochemical properties of dynamised solutions prepared from active ingredients of plant and mineral origin,[10] biological,[11,12] and synthetic[13] have been studied in centesimal potentials,[14] decimals,[15] and 50 millesimal.[16] These studies indicate that dynamic solutions' electrical conductivity and pH increase as solutions age.

Studies developed by Elia *et al*. suggested that the aging of dynamised solutions implies physicochemical changes capable of modifying electrical conductivity and other properties due to the generation of dissipative structures.[17]

The conductivity results of *Arsenicum album* potencies in glass containers increased after twelve months of study in both storage conditions. The opposite behavior was observed in samples in plastic packages [Figure 1]. The results corroborate studies of the electrical conductivity of ultra-diluted aqueous and hydroalcoholic solutions conducted by Holandino *et al*.[18]

Later studies also described changes in the electrical conductivity of dynamised solutions, especially in the first seven days after dynamising the solutions. The most significant changes were observed in the presence of the ionic active ingredient.[13,19,20]

One of the theories described in the literature is that of "water memory," which suggests the ability of water to store memory of the originally solubilised substance and associates the transmission of this memory to the succussion process since in the absence of agitations, the biological effect seems to be significantly influenced.[21,22] Studies by Montagnier *et al*. indicate that water can acquire electromagnetic properties from dynamisation.[23]

In addition to the "memory of water," the literature also describes the "silica hypothesis" involved with the medicinal effect of homoeopathic solutions.[24] According to this hypothesis, glass-manipulated drugs have silicates involved with drug activity. This theory signals the possibility of succussion releasing monomers of silic acid in the solution, which the catalytic action of the mother tincture could polymerize. At powers >12C, the silicates would exert catalytic activity.[24]

In this context, silica's participation in forming water agglomerations and consequently in the drug effect has been suggested, justifying the need to always prepare the dynamisations in glass. However, studies have reported positive results triggered by homoeopathic medicines, even when prepared in plastic flasks, suggesting that the use of

glass may not be an absolute requirement for the appearance of the drug effect.[25,26]

Although the density analysis of homoeopathic matrices is not a regulatory requirement,[6] the difference in the composition of the packages and seals could suggest possible ethanol losses by evaporation. The results of the analysis, shown in Table 1, indicate no difference in the density of the samples ($p > 0.05$) when comparing the two packages at different times and under different storage conditions. In this way, the packaging allowed adequate sealing and low vapor permeation, which is already well-established for PET in the case of plastic packaging.

Pharmaceutical packaging is designed to contain a product in a way that does not interact with the environment. Resistance, durability, flexibility, hardness, and biocompatibility are important requirements. Plastics have numerous advantages because they are packaging traditional products used for years in the food industry. These advantages have led plastic to assume a dominant role in the packaging market for pharmaceutical use. Despite this, no plastic material can be compared to Type II glass in terms of its permeability and inertness. The resistance of plastics to light, oxygen, solvents, and heat cannot be compared to glass or metal. Its resistance properties can be improved by adding specific substances or surface treatments. These additives and treatments can be removed from plastic packaging by solvents and, in some cases, by the drugs themselves. Solvents or the drug can also remove polymers of low molecular weight in plastic packaging. This point highlights the importance of packaging for pharmaceutical products made of different materials.[27]

PET is classified as a thermoplastic material that becomes fluid with heat and hardens again on cooling. The material is robust, lightweight, and break resistant. It has good acceptance by consumers and is suitable for dispensing medications that interact less with the packaging. The disadvantage is the inability to withstand heat and unsuitable for autoclave sterilisation. Plastic packaging has been used in liquid oral formulations and is the most used form in pediatric and geriatric products.[28,29]

Microbiological aspects are also considered in the stability of medicines. Microbial contamination of a product can lead to changes in its physical and chemical properties, in addition to featuring a risk of infection for the user. Thus, oral and topical pharmaceutical products (capsules, pills, suspensions, creams, and adhesives) must be subjected to microbial contamination control.[6,8] Solutions with alcohol graduations around 70% (w/w) are bactericidal. However, during storage, the alcohol content could decrease and compromise the microbiological integrity of *Arsenicum album* solutions. The results indicated that the samples remained following the microbiological specification during the twelve months of storage in glass and PET, suggesting the efficiency of both packaging materials.

PET, as primary packaging for homoeopathic medicines, has advantages and disadvantages compared to glass flasks. It is noteworthy that homoeopathic matrices have a shelf life of five years and are usually stored in glass packaging.[30] However, a stability study using PET packaging has yet to be analysed. On the other hand, the main advantage of its use is observed in the dispensing. PET has greater consumer acceptance because it resists to breakage.[31] It would make PET suitable for use, especially for dispensing medications with less interaction with the packaging.

Few studies investigated the influence of amber glass (type II) packages for homoeopathic medicines and compared it with PET packages. A previous study investigated the biological effects of *Arsenicum album* 6C and 200C prepared and stored in these two packages (glass and PET).[7] It was verified that samples prepared in PET showed pro-inflammatory activities, which can be related to organic residuals found in the vials during the agitation process. However, these effects were not observed for glass vials samples.[7]

Another study stored distilled water for 12 hours after dynamising in two packages (amber glass Class II and high-density polyethylene bottles).[32] It was found that higher concentrations of Si, Li, Na, and Mg were in glass vials than in plastic vials. These results could be referred to the theory of silica particulates present in glass vials.[32]

However, there is much to be researched until we reach a consensus about the stability and behavior of dynamic solutions. Especially, PET, being an unconventional material for compounding or storage of homoeopathic products, further similar studies are required before stating it as inert or stable.

Conclusion

The results show that *Arsenicum album* 6C and 30C are stable preparations. It can also be concluded that glass packages are suitable for preparing and packaging dynamised solutions of *Arsenicum album*. PET has also demonstrated the ability to maintain the physicochemical and microbiological stability of the solutions and may be a potential alternative to prepare or store homoeopathic matrices. However, further studies are required to confirm the suitability of PET vials in compounding and storing homoeopathic medicines.

Acknowledgments

The authors would like to thank the Quality Control Laboratory of Farmanguinhos, Fiocruz, for making the use of climatic chambers available. In addition to Dra. Helena Keiko Toma for microbiologic analysis support.

Funding

We thank the Brazilian Association of Homeopathic Pharmacists (Associação Brasileira de Farmacêuticos Homeopatas—ABFH) for partially funding this study.

Conflicts of interest

The authors declare no competing interests.

References

1. Teixeira MZ. "New homeopathic medicines" proposal: A database made available in three free-access bilingual digital books. Rev Assoc Méd Bras (1992) 2021;67:1387-91.
2. Ive EC, Couchman IM, Reddy L. Therapeutic effect of Arsenicum album on leukocytes. Int J Mol Sci 2012;13:3979-87.
3. Resolution of the Collegiate Board (RDC). Establishes the Criteria for Conducting Stability Studies for Active Pharmaceutical Ingredients and Non-biological Drugs, and Makes Other Arrangements. The Resolution RDC 318; 2019. P. 1-27.
4. Brazilian Health Regulatory Agency (ANVISA), Brazil. Brazilian Homeopathic Pharmacopoeia. 3ª ed. Brasília, DF: Brazilian Health Regulatory Agency (ANVISA); 2011.
5. Paumier A, Verre J, Tribolo S, Boujedaini N. Anti-oxidant effect of high dilutions of *Arnica montana*, Arsenicum album, and Lachesis mutus in microglial cells *in vitro*. Dose Response 2022;20;1-7. doi: 10.1177/15593258221103698
6. Instrução Normativa nº 04 de 11 de abril de 2007. Dispõe Sobre o Guia Para a Realização de Estudos de Estabilidade para Medicamentos Dinamizados; 2007.
7. Dalboni LC, Coelho CP, Palombo Pedro RR, Correia MS, De Santana FR, Cardoso TN, *et al*. Biological actions, electrical conductance and silicon-containing microparticles of arsenicum album prepared in plastic and glass vials. Homeopathy 2019;108:12-23.
8. Brazilian Health Regulatory Agency (ANVISA), Brazil. Brazilian Pharmacopea. 6ª ed. Brasília, DF: Brazilian Health Regulatory Agency (ANVISA); 2019.
9. Pharmacopea US. <644> Conductivity Of Solutions. General Chapters. Rockville: United States Pharmacopea; 2021.
10. Fontes C, Oliveira AP, Batista JV, Martins C, Rossi A, Yapuchura ER, *et al*. Physicochemical properties of zinc and lactose in solid mixtures: Influence of trituration process. Homeopathy 2022;111:164-75.
11. Cajueiro AP, Goma EP, Dos Santos HA, Almeida Rodrigues I, Toma HK, Araújo SM, *et al*. Homeopathic medicines cause Th1 predominance and induce spleen and megakaryocytes changes in BALB/c mice infected with Leishmania infantum. Cytokine 2017;95:97-101.
12. Holandino C, Siqueira CM, Homsani F, Paiva J, Veiga VF, Oliveira AP. Therapeutic potential of nosodes: Challenges, translationality and perspectives. In: Transdisciplinarity and translationality in high dilution research: Signals and images GIRI Series. 1. 1st ed. Cambridge: Cambridge Scholars Publishing; 2019. p. 244-65.
13. Holandino C, Leal FD, De Oliveira Barcellos B, Campos MA, Oliveira R, Feo Da Veiga V, *et al*. Mechanical versus handmade succussions: A physical chemistry comparison. In: Bonamin LV, editor. Signals and Images: Contributions and Contradictions about High Dilution Research. Dordrecht: Springer Netherlands; 2008. p. 37-48.
14. De Oliveira SM, De Oliveira CC, Abud AP, Guimarães FS, Bernardi RP, Coletto EL, *et al*. Mercurius solubilis: Actions on macrophages. Homeopathy 2011;100:228-36.
15. Siqueira CM, Costa BG, Amorim AM, Gonçalves M, Veiga VF, Branco MC, *et al*. H3N2 homeopathic influenza virus solution modifies cellular and biochemical aspects of MDCK and J774G8 cell lines. Homeopathy 2013;102:31-40.
16. Garcia S, Harduim RC, Homsani F, Zacharias CR, Kurster R, Holandino C. Physical chemical and citotoxic evaluation of highly diluted solutions of *Euphorbia tirucalli* L. prepared through the fifty milesimal homeopathic method. Int J High Dilution Res 2010;9:63-73.
17. Elia V, Napoli E, Germano R. The 'memory of water': An almost deciphered enigma. Dissipative structures in extremely dilute aqueous solutions. Homeopathy 2007;96:163-9.
18. Holandino C, Harduim RC, Veiga VF, Garcia S, Zacharias CR. Modeling physical-chemical properties of high dilutions: An electrical condutivity study. Int J High Dilution Res 2008;7:165-73.
19. Elia V. Physico-chemical properties of perturbed water: Facts and enigmas. Int J High Dilution Res 2012;11:110-2.
20. Elia V, Baiano S, Duro I, Napoli E, Niccoli M, Nonatelli L. Permanent physico-chemical properties of extremely diluted aqueous solutions of homeopathic medicines. Homeopathy 2004;93:144-50.
21. Rey L. Can low-temperature thermoluminescence cast light on the nature of ultra-high dilutions? Homeopathy 2007;96:170-4.
22. Tournier A, Würtenberger S, Klein SD, Baumgartner S. Phyiscochemical investigations of homeopathic preparations: A systematic review and bibliometric analysis-part 3. J Altern Complement Med 2021;27:45-57.
23. Montagnier L, Aissa J, Giudice ED, Lavallee C, Tedeschi A, Vitiello G. DNA waves and water. J Phy Conf Ser 2011;306:012007.
24. Anick DJ, Ives JA. The silica hypothesis for homeopathy: Physical chemistry. Homeopathy 2007;96:189-95.
25. Chirumbolo S, Brizzi M, Ortolani R, Vella A, Bellavite P. Inhibition of CD203c membrane up-regulation in human basophils by high dilutions of histamine: A controlled replication study. Inflamm Res 2009;58:755-64.
26. Bellavite P, Magnani P, Zanolin E, Conforti A. Homeopathic doses of Gelsemium sempervirens improve the behavior of mice in response to novel environments. Evid Based Complement Alternat Med 2011;2011:362517.
27. Bauer JE. Pharmaceutical Packaging Handbook. USA: Informa Healthcare, Inc.; 2009. p. 610.
28. Das PS, Saha P, Krishan, Das R. Pharmaceutical packaging technology: A brief outline. Res J Pharm Dosage Forms Technol 2018;10:16-21.
29. Pegu L, Chasta P, Chandrul KK. Pharmaceutical packaging technology. Int J Trend Sci Res Dev 2019;3:1747-55.
30. Valavan R, Cesar A. Current status of expiry of homeopathic medicines in Brazil, Germany, India and the United States: A critical review. Homeopathy 2019;108:232-9.
31. Selke SE, Hernandez RJ. Packaging: Polymers for containers. In: Buschow KH, Cahn RW, Flemings MC, Ilschner B, Kramer EJ, Mahajan S, *et al*., editors. Encyclopedia of Materials: Science and Technology. Oxford: Elsevier; 2001. p. 6646-52.
32. Witt CM, Lüdtke R, Weisshuhn TE, Quint P, Willich SN. The role of trace elements in homeopathic preparation and the influence of container material, storage duration, and potentisation. Forsch Komplementärmed 2006;13:15-21.

Étude de stabilité de l'Arsenicum album dynamisé homéopathiquement dans du verre ambré hydrolytique de classe II et du polyéthylène téréphtalate haute densité sur douze mois

Contexte: La Pharmacopée homéopathique brésilienne (BHP) recommande d'utiliser du verre ambré de type 1, II ou III comme récipient principal pour le stockage des médicaments liquides homéopathiques. Bien que le verre présente des avantages, il présente certains inconvénients, tels que la fragilité, le poids et la présence de particules de silicate, qui ont été décrites comme des composants critiques impliqués dans la génération de microparticules de silicium détectées dans les solutions homéopathiques. **Objectifs:** La présente étude visait à étudier la stabilité des solutions hydroalcooliques d'Arsenicum album dynamisées dans deux flacons différents : verre ambré hydrolytique de classe II et polyéthylène téréphtalate haute densité (PET) ambré. Français : **Matériel et méthodes:** Deux concentrations d'*Arsenicum album*, 6C et 30C, ont été préparées selon BHP. Les solutions ont été stockées dans des flacons en verre et en PET ambré. L'étude de stabilité a été réalisée dans une chambre climatique selon les conditions décrites dans le recueil officiel brésilien pendant douze mois. Au cours de l'étude, les échantillons ont été évalués par densité, pH, réfractométrie et conductivité. La pureté microbiologique a été évaluée au début et à la fin de l'étude de stabilité. **Résultats:** Aucune différence statistique n'a été observée dans la densité des échantillons dans les deux conditions et dans les emballages. Le pH de tous les échantillons est resté autour de 6 et la réfractométrie autour de 1,363, suggérant leur stabilité chimique. Les analyses microbiologiques n'ont montré aucun micro-organisme pathogène après douze mois de stockage de tous les échantillons. **Conclusion:** Les résultats ont montré qu'*Arsenicum album* 6C et 30C sont des préparations stables. On peut également conclure que les bouteilles en verre sont adaptées au stockage des solutions dynamisées d'*Arsenicum album*. Les flacons en PET ont permis de maintenir la stabilité physicochimique et microbiologique des solutions et peuvent constituer une alternative potentielle pour la préparation ou le stockage de matrices homéopathiques. Cependant, des études supplémentaires sont nécessaires pour confirmer l'adéquation des flacons en PET à la préparation et au stockage de médicaments homéopathiques

Stabilitätsstudie von homöopathisch potenziertem *Arsenicum album* in hydrolytischem Braunglas der Klasse II und hochdichtem Polyethylenterephthalat über zwölf Monate

Hintergrund: Das brasilianische Homöopathische Arzneibuch (BHP) empfiehlt die Verwendung von Braunglas der Klasse I, II oder III als primären Behälter zur Aufbewahrung homöopathischer flüssiger Arzneimittel. Obwohl Glas Vorteile hat, gibt es auch einige Nachteile, wie Zerbrechlichkeit, Gewicht und das Vorhandensein von Silikatpartikeln, die als kritische Komponenten beschrieben wurden, die an der Bildung von Siliziummikropartikeln beteiligt sind, die in homöopathischen Lösungen nachgewiesen werden. **Ziele:** Ziel der vorliegenden Studie war es, die Stabilität von hydroalkoholischen Lösungen von dynamisiertem *Arsenicum album* in zwei verschiedenen Fläschchen zu untersuchen: hydrolytischem Braunglas der Klasse II und bernsteinfarbenem hochdichtem Polyethylenterephthalat (PET). **Materialien und Methoden:** Zwei Potenzen von *Arsenicum album*, 6C und 30C, wurden gemäß BHP hergestellt. Die Lösungen wurden in Glas- und bernsteinfarbenen PET-Flaschen aufbewahrt. Die Stabilitätsstudie wurde zwölf Monate lang in einer Klimakammer gemäß den im offiziellen brasilianischen Kompendium beschriebenen Bedingungen durchgeführt. Während der Studie wurden die Proben nach Dichte, pH-Wert, Refraktometrie und Leitfähigkeit bewertet. Die mikrobiologische Reinheit wurde zu Beginn und am Ende der Stabilitätsstudie bewertet. **Ergebnisse:** Es wurden keine statistischen Unterschiede in der Dichte der Proben in beiden Zuständen und Verpackungen beobachtet. Der pH-Wert aller Proben blieb bei etwa 6 und die Refraktometrie bei etwa 1,363, was auf ihre chemische Stabilität hindeutet. Die mikrobiologischen Tests zeigten nach zwölfmonatiger Lagerung aller Proben keine pathogenen Mikroorganismen. **Schlussfolgerung:** Die Ergebnisse zeigten, dass *Arsenicum album* 6C und 30C stabile Präparate sind. Es kann auch der Schluss gezogen werden, dass Glasflaschen zur Lagerung dynamisierter Lösungen von *Arsenicum album* geeignet sind. PET-Flaschen behielten die physikochemischen und mikrobiologischen Stabilität der Lösungen bei und können eine potenzielle Alternative zur Herstellung oder Lagerung homöopathischer Matrizen sein. Es sind jedoch weitere Studien erforderlich, um die Eignung von PET-Fläschchen für die Herstellung und Lagerung homöopathischer Arzneimittel zu bestätigen.

बारह महीनों में हाइड्रोलाइटिक एम्बर ग्लास क्लास II और हाई-डेंसिटी पॉलीइथाइलीन टेरेफ्थेलेट में होम्योपैथिक रूप से पोटेन्टाइज्ड *आर्सेनिकम एल्बम* की स्थिरता का अध्ययन

पृष्ठभूमि: ब्राजीलियन होम्योपैथिक फार्माकोपिया (BHP) होम्योपैथिक तरल दवाओं के भंडारण के लिए प्राथमिक कंटेनर के रूप में ग्लास एम्बर टाइप I, II या III का उपयोग करने की सलाह देता है। हालाँकि ग्लास के फायदे हैं, लेकिन कुछ नुकसान भी हैं, जैसे कि नाजुकता, वजन और सिलिकेट कणों की उपस्थिति, जिन्हें होम्योपैथिक घोल में पाए जाने वाले सिलिकॉन माइक्रोपार्टिकल्स की पीढ़ी से जुड़े महत्वपूर्ण घटकों के रूप में वर्णित किया गया है। **उद्देश्य:** वर्तमान अध्ययन का उद्देश्य दो अलग-अलग शीशियों (क्लास II हाइड्रोलाइटिक एम्बर ग्लास और एम्बर हाई-डेंसिटी पॉलीइथाइलीन टेरेफ्थेलेट (PET)) में डाइनामाईइज्ड *आर्सेनिकम एल्बम* के हाइड्रोअल्कोहोलिक घोल की स्थिरता की जाँच करना था। **सामग्री और विधियाँ:** BHP के अनुसार आर्सेनिकम एल्बम की दो पोटन्सी, 6C और 30C, तैयार की गईं। घोलों को कांच और पीईटी एम्बर फ्लास्क में संग्रहित किया गया। स्थिरता अध्ययन ब्राजील के आधिकारिक संग्रह में वर्णित स्थितियों के अनुसार एक जलवायु कक्ष में बारह महीनों के लिए किया गया। अध्ययन के दौरान, नमूनों का घनत्व, पीएच, रिफ्रेक्टोमेट्री और चालकता द्वारा मूल्यांकन

किया गया। स्थिरता अध्ययन की शुरुआत और अंत में सूक्ष्मजीवविज्ञानी शुद्धता का मूल्यांकन किया गया। **परिणाम:** दोनों स्थितियों और पैकेजों में नमूनों के घनत्व में कोई सांख्यिकीय अंतर नहीं देखा गया। सभी नमूनों का पीएच 6 के आसपास और रिफ्रेक्टोमेट्री 1.363 के आसपास रहा, जो उनकी रासायनिक स्थिरता का सुझाव देता है। सभी नमूनों को बारह महीने तक संग्रहीत करने के बाद सूक्ष्मजीवविज्ञानी परख में कोई रोगजनक सूक्ष्मजीव नहीं पाए गए। **निष्कर्ष:** परिणामों से पता चला कि *आर्सेनिकम एल्बम* 6C और 30C स्थिर तैयारी हैं। यह भी निष्कर्ष निकाला जा सकता है कि कांच की बोतलें *आर्सेनिकम एल्बम* के डाइनामाईज्ड घोल को संग्रहीत करने के लिए उपयुक्त हैं। पीईटी बोतलों ने घोल की भौतिक और सूक्ष्मजीवविज्ञानी स्थिरता बनाए रखी इसलिए होम्योपैथिक मैट्रिक्स को तैयार करने या संग्रहीत करने के लिए यह एक संभावित विकल्प हो सकता है। हालांकि, होम्योपैथिक दवाओं के मिश्रण और भंडारण में पीईटी शीशियों की उपयुक्तता की पुष्टि के लिए आगे के अध्ययन की आवश्यकता है।

Estudio de estabilidad de Arsenicum album potenciado homeopáticamente en vidrio ámbar hidrolítico de clase II y tereftalato de polietileno de alta densidad durante doce meses

Antecedentes: La Farmacopea Homeopática Brasileña (BHP) recomienda utilizar vidrio ámbar de tipo I, II o III como recipiente principal para almacenar medicamentos líquidos homeopáticos. Aunque el vidrio tiene ventajas, existen algunas desventajas, como la fragilidad, el peso y la presencia de partículas de silicato, que se han descrito como componentes críticos involucrados en la generación de micropartículas de silicio detectadas en soluciones homeopáticas. **Objetivos:** El presente estudio tuvo como objetivo investigar la estabilidad de soluciones hidroalcohólicas de Arsenicum album dinamizadas en dos viales diferentes: vidrio ámbar hidrolítico clase II y tereftalato de polietileno de alta densidad (PET) ámbar. **Materiales y métodos:** Se prepararon dos potencias de *Arsenicum album*, 6C y 30C, según BHP. Las soluciones se almacenaron en frascos ámbar de vidrio y PET. El estudio de estabilidad se realizó en una cámara climática de acuerdo con las condiciones descritas en el compendio oficial brasileño durante doce meses. Durante el estudio, las muestras se evaluaron por densidad, pH, refractometría y conductividad. La pureza microbiológica se evaluó al inicio y al final del estudio de estabilidad. **Resultados:** No se observaron diferencias estadísticas en la densidad de las muestras en ambas condiciones y paquetes. El pH de todas las muestras se mantuvo alrededor de 6 y la refractometría alrededor de 1,363, lo que sugiere su estabilidad química. Los ensayos microbiológicos no mostraron microorganismos patógenos después de doce meses de almacenamiento de todas las muestras. **Conclusión:** Los resultados mostraron que *Arsenicum album* 6C y 30C son preparaciones estables. También se puede concluir que las botellas de vidrio son adecuadas para almacenar soluciones dinamizadas de *Arsenicum album*. Las botellas de PET mantuvieron la estabilidad fisicoquímica y microbiológica de las soluciones y pueden ser una alternativa potencial para preparar o almacenar matrices homeopáticas. Sin embargo, se requieren más estudios para confirmar la idoneidad de los viales de PET para la preparación y el almacenamiento de medicamentos homeopáticos.

十二个月内，在水解琥珀色玻璃 II 类和高密度聚对苯二甲酸乙二醇酯中，对 势 法增效的白砷 行 定性研究

背景：巴西 势 法 典（BHP）建 使用琥珀色玻璃 I、II 或 III 型作 存 势 法液体 物的主要容器。尽管玻璃具有优点，但也存在一些缺点，例如易碎、重量大以及存在硅酸 粒，这些 粒被 是与在 势 法溶液中 到的硅微粒的生成有 的 成分。目的：**本研究旨在 查在两种不同小瓶中增效的白砷水醇溶液的定性：**水解琥珀色玻璃 II 类和琥珀色高密度聚对苯二甲酸乙二醇酯（PET）。材料和方法：根据 BHP 制 两种效力的白砷，6C 和 30C。溶液 存在玻璃和 PET 琥珀色 瓶中。 定性研究在气候室中根据巴西官方概要中描述的条件 行了十二个月的 定性研究。在研究期 ，通过密度、pH、折射法和 率 估样品。在 定性研究 始和 束时 估微生物 度。 **果：**在两种条件和包 中，样品的密度没有 学差异。所有样品的 pH 值保持在 6 左右，折射法保持在 1.363 左右，表明其化学 定性。微生物学 定表明，**所有样品 存十二个月后均未 致病微生物。 ：** 果表明，*Arsenicum album* 6C 和 30C 是 定的制 。还可以得出 ，玻璃瓶适合 存 *Arsenicum album* 的动 溶液。PET 瓶保持了溶液的物理化学和微生物 定性，可以作 制 或 存 势 法基 的潜在替代品。然而，还需要 一步研究来确 PET 小瓶是否适合配制和 存 势 法 物。