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ACCELERATED STABILITY STUDY OF BENZOYL PEROXIDE IN MAGISTRAL FORMULATIONS

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ABSTRACT

In acne treatment, Benzoyl Peroxide is often used. Can be magistral manipulated in gel and cream forms, but his chemical instability makes hard to maintain efficiency and stability of product. The objective of this article is to verify manipulated Benzoyl Peroxide at 2.5% (w/w) stability in gel and cream forms, evaluating formulation in Brazilian National Formulary 2nd Edition by stress test and degradation profile in 0, 7, 30, 60 and 90 days. Was measured Benzoyl Peroxide and Benzoic Acid dosage determination and his kinetics, physical-chemical (pH, viscosity) and organoleptic aspects (color, odor and appearance) in ambient, sunlight, refrigerator and oven conditions. To analyses, it was formulated gel and cream Benzoyl Peroxide at 2.5% (w/w), tested in iodometric and acid-base titration to Benzoyl Peroxide and Benzoic Acid dosage determination, Brookfield viscometer to viscosity, pH meter to pH, and organoleptic evaluation in color, odor and appearance, respectively. Organoleptic and physical-chemical analyses were not statistic significant ($p>0.05$) but dosages were significant and proved superior stability in cream than gel sample. Are requested further studies to develop more stable formulations for this product.

Keywords: Benzoyl Peroxide Gel/Cream, Benzoic Acid, Stress Test, Manipulated Stability Formulations.

INTRODUCTION

Acne vulgaris is a common skin pathology, being a chronic pilosebaceous inflammation. There is no known cure, but with medication is controllable, starting with topic treatment. Most used agents are topics retinoics, including Benzoyl Peroxide and he has inflammatory and comedogenic least effects [1,2].

Benzoyl Peroxide have as action mechanism free radicals reaction by slow release of oxygen, reduction of bacterial lypases and sebostatic activity [3]. Because of it, Benzoyl Peroxide is unstable, very reactive and has a poor aqueous solubility, making him being common formulated in topical suspensions. Is found in gel and cream forms, in a 1-10% (w/w) concentration, to avoid much skin irritation and there is no evidence of systematic toxicity [4,5].

Stability of magistral formulations are not uniform, due to many factors interference such as temperature, light, oxygen, humidity, physical-chemical incompatibilities between peroxide and his emollient. Any modification in pharmacological structure or conditions affects quality of formulated product, leaving the established standards. Knowledge of stability behavior in different magisterial formulations is fundamental in order to ensure final quality [6].

Gel and cream in compounding pharmacies bases their formulations in Brazilian National Formulary [7]. They contain different types of solvents and vehicles, which can affect directly on the stability of Benzoyl Peroxide in pharmaceutical formulations. The chemical instability of Benzoyl Peroxide makes it very difficult to make a resistant and stable formula [4].

The objective of this article is to verify manipulated Benzoyl Peroxide at 2.5% (w/w) stability in gel and cream forms, evaluating the formulation in Brazilian National Formulary 2nd Edition [7] by stress test and degradation profile in 0, 7, 30, 60 and 90 days. Was measured the Benzoyl Peroxide and Benzoic Acid dosage determination and his kinetics, physical-chemical (pH, viscosity) and organoleptic aspects (color, odor and appearance) in ambient, sunlight, refrigerator and oven conditions.

MATERIALS AND METHODS

Dosage determination, formulation details,

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physical-chemical and organoleptic aspects test were used to investigate alterations of formulations under different conditions using specific guides from ANVISA (National Sanitary Vigilance Agency), as established by Brazilian's Laws. For dosage determination was to be used Brazilian Pharmacopoeia 5th Edition [8] (RDC 49/2010), but since this and previous edition did not show how to measure Benzoyl Peroxide and Benzoic Acid, was used European Pharmacopoeia 8th Edition [9]. For gel and cream formulations was used Brazilian National Formulary 2nd Edition [7] (RDC 222/2005) and for physical-chemical and organoleptic aspects assays Cosmetic Products Stability Guide [6] (RDC 29/2005).

This experiment is to observe gel and cream 2.5 % (w/w) Benzoyl Peroxide stress test conducted with following analysis: dosage determination of Benzoyl Peroxide degradation and Benzoic Acid formation product and their kinetics in duplicate. Physical-chemical and organoleptic aspects stability by pH, viscosity, odor, color and appearance in one sample. To perform accelerated stability test, gel and cream were at each of following conditions: ambient, sunlight, refrigerator, oven and measure in 0, 7, 30, 60, 90 days. They were withdrawn and placed at room temperature for 30 minutes from refrigerator and oven to eliminate any temperature interference before carrying out any assay.

Formulation

Gel (100g) [7]

Phase A: Paraben 3.3g; Glycerin 5.0g; Disodium Edetate 0.1 g; Alcohol 70% 100 g.

Phase B: Carbopol 940 1.0g

Phase C: Triethanolamine 0.6g

In a recipient, dismiss Phase B components previously mixed in Phase A, waiting for complete dispersion of polymer. Add Phase C under agitation. Verify pH and correct to 5.5-6.0.

To add Benzoyl Peroxide at 2.5%, use geometric addiction of 195 g of base and 5 g of Benzoyl Peroxide.

Cream (100g) [7]:

Water Phase (A): Disodium Edetate 0.10g; Glycerin 3g; Water 100 g.

Oil Phase (B): Non-Ionic Auto Emulsifying Wax 10.00g; Liquid Paraffin 2.00g; Non-Ionic Auto Emulsifying Wax 200 E 2.1g; Glyceryl Stearate 0.9g; Butyl-Lathed Hydroxytoluene 0.05g.

Complementary Phase (C): Preservative 0.60g.

Heat separately Phase B and Phase A under 80 ºC. In slow agitation add Phase A in Phase B until 40 ºC, and add Phase C. Verify pH and correct to 5.5-6.0.

To add Benzoyl Peroxide at 2.5%, use geometric addiction of 195 g of base and 5g of Benzoyl Peroxide.

Benzoyl Peroxide dosage determination [9]: Dissolve 5 g of gel or cream with 50 ml of acetone. Added to a sufficient amount to 100 ml of acetone and remove 10 ml of mixture. Add 25 ml of 20% potassium iodide solution (w/v) and mix them. Close the bottle and keep in a place protected from light for 15 minutes. After this add more 25 ml of acetone and titrate with 0.1 M SV sodium thiosulphate using starch as indicator. Endpoint of titration is a transparent color of the sample.

Benzoic Acid dosage determination [9]: Dissolve 0.2 g of gel or cream in 20 ml of ethanol and titrate with sodium hydroxide 0.1M SV, using phenol red SI until violet color, which indicates the endpoint.

pH potentiometric determination [6]: Calibration of pH meter was in acidic and basic condition in 4.0 and 7.0 pH. Placed the electrode and measure pH samples.

Viscosity [6]: Was used the Brookfield viscometer. For measure, leveled off viscometer and chosen an appropriate spindle for selected samples. When connected to the device, spindle had immersed until his mark and after was regulated the speed.

Organoleptic assay [6]: Verified organoleptic characteristics as color, odor, appearance and samples were classified as no, little and much alterations.

Statistical Analysis

For analysis results, it was used SPSS Statistics 23 Version Program (IBM). Shapiro-Wilk normality test indicated distribution data of physical-chemical and organoleptic aspects. Most of it were non-parametric and some were parametric. For this, it was used the Kruskall-Wallis. Were used Mann-Whitney and T-student test to observe differences between groups separately. Kolmogorov-Sminorv showed Benzoyl Peroxide and Benzoic Acid dosage determination normality and were parametric. To them was used factorial 2x5x4 ANOVA test with Tukey Post-Hoc. P-value considerate statistically significant was $p<0.05$ and was used a confidence interval of 95% of data results.

RESULTS

Stress test

Unfortunately, all assays in all conditions tested were no statistically significant between days and conditions.

Benzoyl Peroxide and Benzoic Acid Profiles in Gel

Benzoyl Peroxide Gel Degradation and Benzoic Acid Formation results are available in Table [1], showing their loss and gains (in grams) plus their standard derivations in different conditions. All Graphics performance degradation and formation are available in Appendix 1 as figures. Heat conditions as oven Fig [1] and [2] and sunlight Fig [3] and [4] had worst quality performance; ambient Fig [5] and [6] and refrigerator Fig [7] and [8] had a better resistance with gel, although Benzoyl Peroxide degradation and Benzoic Acid formation were still present. All of them have a first order kinetics rate.

A gel formulation of Benzoyl Peroxide study showed that following solvents: PEG 400, ethanol and propylene glycol are degraded in 1.4, 29 and 53 days at 400°C, recommending not to sell this in market. In the same

study, a formulation containing Carbomer lost 6% of Benzoyl Peroxide at 200°C and 21.7% at 400°C in 3 months, being less stable than an aqueous gel with hydroxyl propyl celluloses, who lost less than 2% at the highest temperature analyzed, by iodometric assay [10]. When there is a comparison between Benzoyl Peroxide gel formulations, the type of surfactant have an impact on degradation profile. Amphoteric Surfactant as Sodium Cocoamphoacetate and Disodium Cocoamphoacetate leads to more than 99.5% of degradation within 1 month at 400°C. With Non-Ionic Surfactant as Sucrose Laurate and Decyl Glucoside were degraded 0.04% and 5.3%, respectively in 2 months at same temperature. Mild Ionic Surfactant as Zinc Coceth Sulfate, Sodium Cocoyl Isethionate and Sodium Methyl Cocoyl Isethionate lost 5.3%, 0% and 9.9% of Benzoyl Peroxide in same conditions [11].

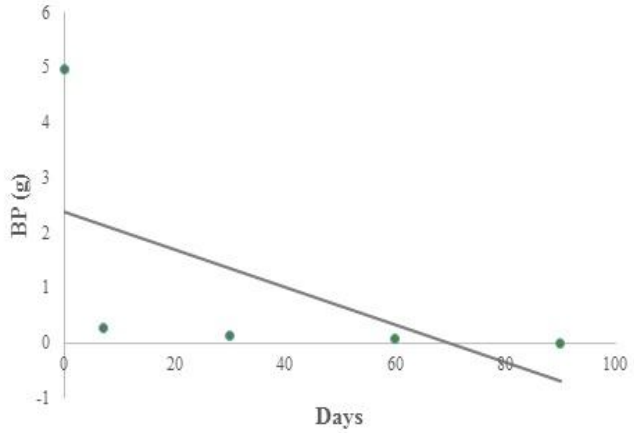
Benzoyl Peroxide and Benzoic Acid Profiles in Cream

Benzoyl Peroxide Cream Degradation and Benzoic Acid Formation results are available in Table [2] showing their loss and gains (in grams) plus their standard derivations in different conditions. Cream showed a more resistant formula than gel, but still degrading more Benzoyl Peroxide and forming Benzoic Acid at oven Fig [9] and [10] and sunlight Fig [11] and [12] conditions.

In foam formulation, addition of glycerin and Sodium PCA slows down degradation profile, only 1.94% and formulations without them degrades between 7.26-13.2% in 2 weeks at 400°C [19]. At the same study, foam formulations with 6% of oil plus emollient degraded between 1.2-1.8% of Benzoyl Peroxide and oil-free formulation did not degrade in 6 months at 300°C [12]. In Cream gels, a speed stability test indicates within 2 months did not had alterations in Benzoyl Peroxide expect content at ambient and 400°C temperature [13]. Benzoyl Peroxide cream lotion mix at 10% with Tretinoin gel 0.025%, under ambient degraded 80% and at visible sunlight was 95% both in 24h [14].

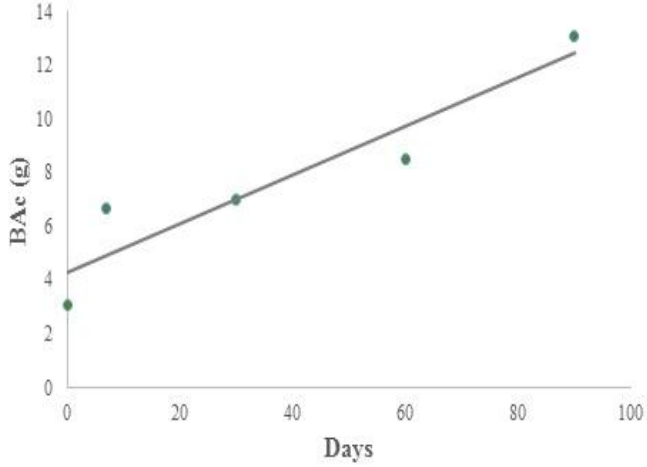
Ambient Fig [13] and [14] and refrigerator Fig [15] and [16] conditions had a minor degradation at all conditions, however still maintains Benzoyl Peroxide degradation and Benzoic Acid formation. All of them have a first order kinetics rate.

Fig 1. Benzoyl Peroxide Oven Gel Degradation. First order reaction.



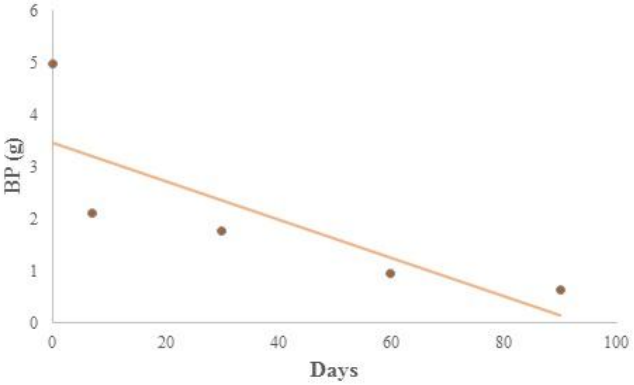
Benzoyl Peroxide = -0.0342x (number of days) + 2.3709. R^2 = 0.3493.

Fig 2. Benzoic Acid Oven Gel Formation



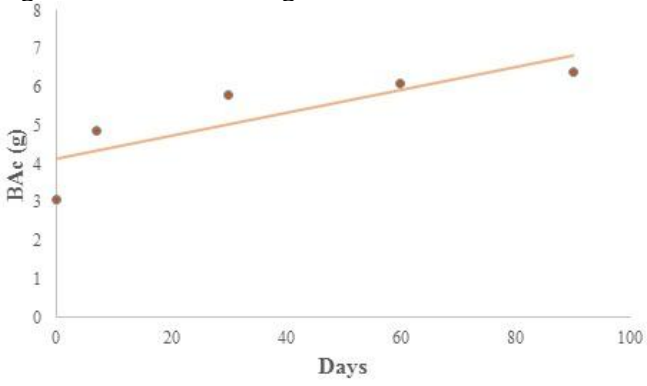
First order reaction: Benzoic Acid = 0.0907x (number of days) + 4.2946. R^2 = 0.876.

Fig 3. Benzoyl Peroxide Sunlight Gel Degradation.



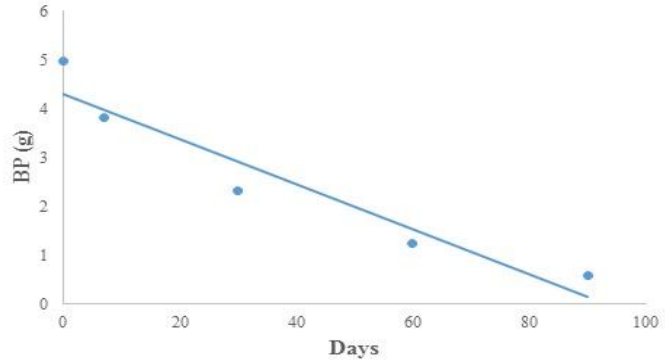
First order reaction: Benzoyl Peroxide = -0.0369x (number of days) + 3.4649. R^2 = 0.648.

Fig 4. Benzoic Acid Sunlight Gel Formation



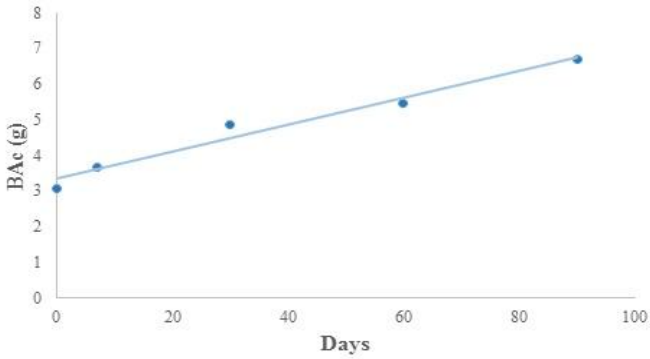
First order reaction: Benzoic Acid = 0.03x (number of days) + 4.1258. R^2 = 0,6929

Fig 5. Benzoyl Peroxide Ambient Gel Degradation



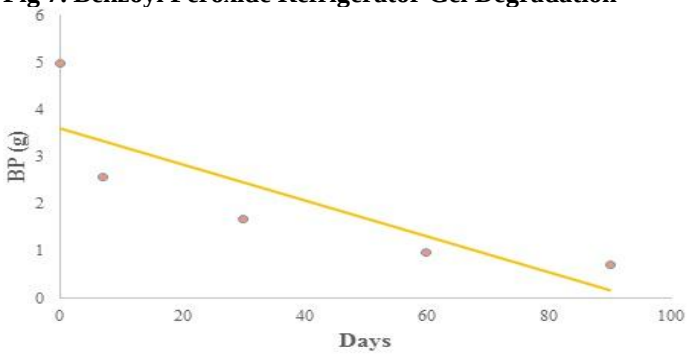
First order reaction: Benzoyl Peroxide = -0.0462x (number of days) + 4.3137. R^2 = 0.9151

Fig 6. Benzoic Acid Ambient Gel Formation.



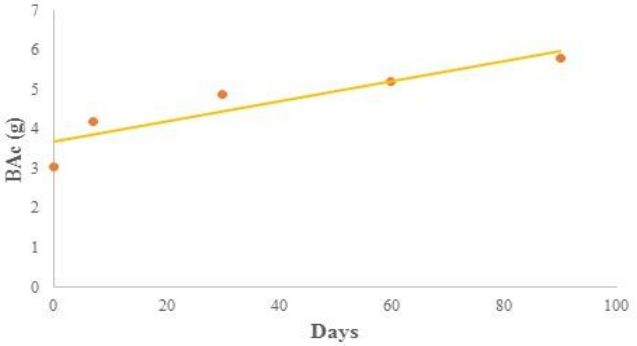
First order reaction: Benzoic Acid = 0.038x (number of days) + 3.3308. R^2 = 0.9675

Fig 7. Benzoyl Peroxide Refrigerator Gel Degradation



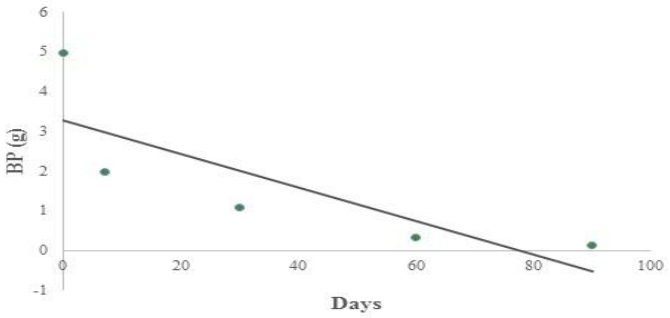
First order reaction: Benzoyl Peroxide = -0.0383x (number of days) + 3.6122. R^2 = 0.7009

Fig 8. Benzoic Acid Refrigerator Gel Formation



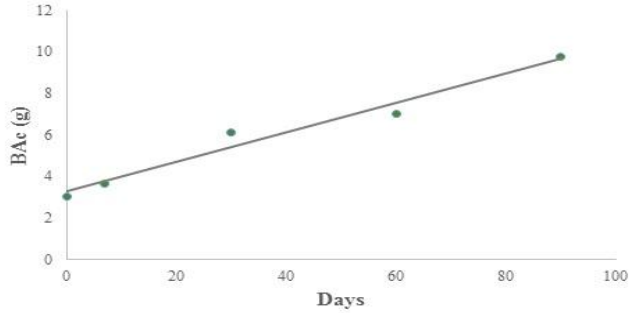
First order reaction: Benzoic Acid = 0.0256x (number of days) + 3.6683. R^2 = 0.8343.

Fig 9. Benzoyl Peroxide Oven Cream Degradation



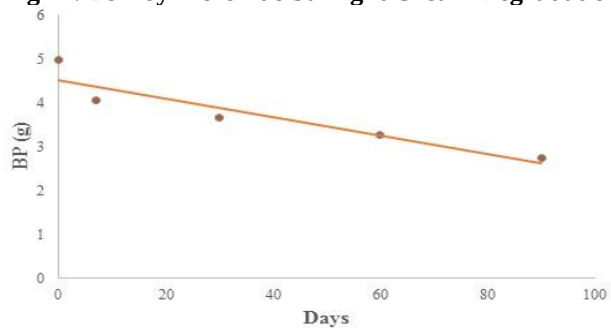
First order reaction: Benzoyl Peroxide = -0.0425x (number of days) + 3.2881. R^2 = 0,6552

Fig 10. Benzoic Acid Oven Cream Formation



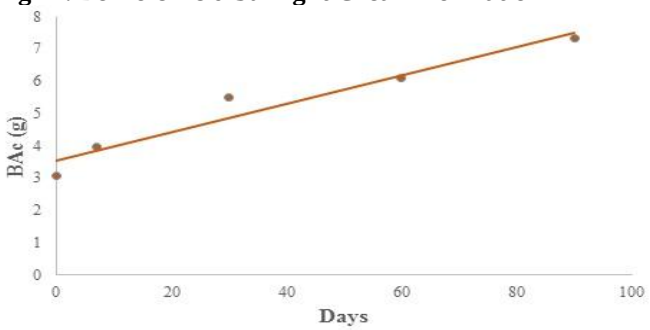
First order reaction: Benzoic Acid = 0.071x (number of days) + 3.2624. R^2 = 0.9721

Fig 11. Benzoyl Peroxide Sunlight Cream Degradation



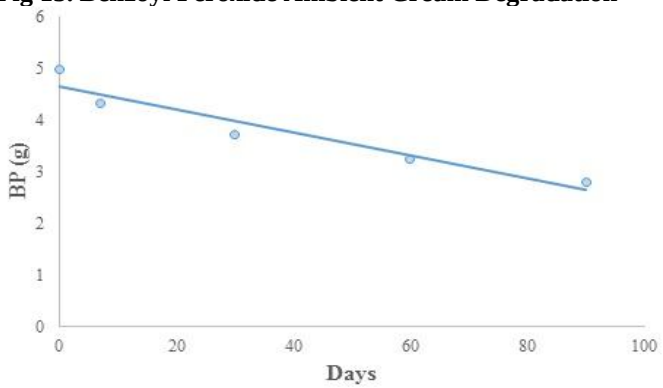
First order reaction: Benzoyl Peroxide = -0.0211x (number of days) + 4.5257. R^2 = 0,8699

Fig 12. Benzoic Acid Sunlight Cream Formation



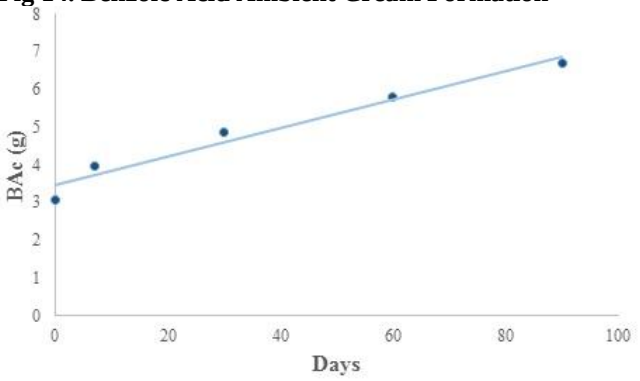
First order reaction: Benzoic Acid = 0.0438x (number of days) + 3.5451. R^2 = 0.9404

Fig 13. Benzoyl Peroxide Ambient Cream Degradation



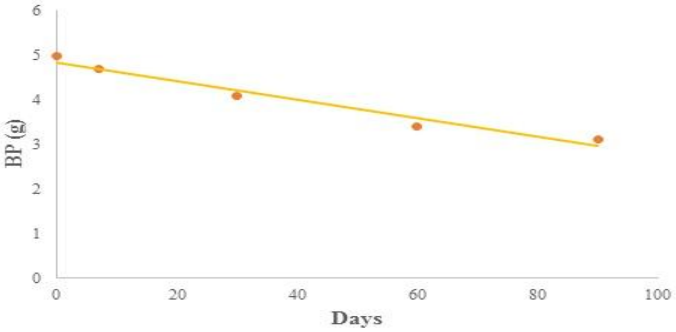
First order reaction: Benzoyl Peroxide = -0.0223x (number of days) + 4.643. R^2 = 0.9249

Fig 14. Benzoic Acid Ambient Cream Formation



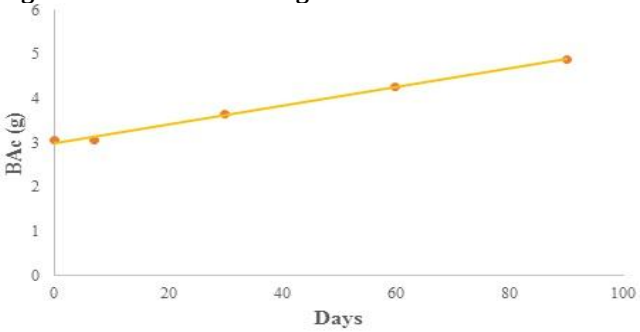
First order reaction: Benzoic Acid = 0.0377x (number of days) + 3.4683. R^2 = 0.9603

Fig 15. Benzoyl Peroxide Refrigerator Cream Degradation



First order reaction: Benzoyl Peroxide = -0,0209x (number of days) + 4.8378. R^2 = 0.9641

Fig 16. Benzoic Acid Refrigerator Cream Formation.



First order reaction: Benzoic Acid = 0.0211x (number of days) + 2.9929. R^2 = 0.9949

Fig 17. Benzoyl Peroxide molecular structure

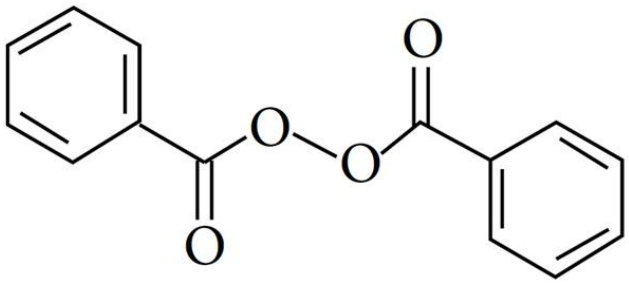


Fig 18. Benzoyl Peroxide chain reaction

(a) $(C_6H_5COO)_2 \rightarrow 2C_6H_5COO^{\bullet}$

(b) $C_6H_5COO^{\bullet} + RH \rightarrow C_6H_5COOH + R^{\bullet}(RH \text{ is solvent})$

(c) $(C_6H_5COO)_2 + R^{\bullet} \rightarrow C_6H_5COOR + C_6H_5COO^{\bullet}$

(d) $R^{\bullet} + R^{\bullet}(\text{or}C_6H_5COO^{\bullet}) \rightarrow \text{termination}$

Fig 19. Benzoic Acid molecular structure

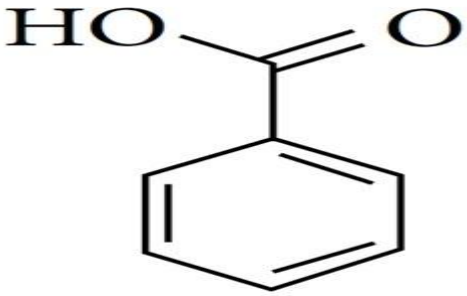


Fig 20. Kinetic rate degradation between solvent and solvent radical termination4

$$\text{Rate} = k_{2p2}(k_i/2k_{t3})^{1/2}\,[BP]^{3/2}$$

Fig 21. Kinetic rate degradation between solvent and benzene radical termination4

$$\text{Rate} = (k_i k_{2p1} k_{2p2}/k_{t2})^{1/2}\,[S]^{1/2}[BP]$$

Table 1. Benzoyl peroxide and benzoic acid gel degradation under tested conditions

Days	Ambient		Sunlight		Refrigerator		Oven	
	BP (g)	BA (g)	BP (g)	BA (g)	BP (g)	BA (g)	BP (g)	BA (g)
0	4.98±0*	3.05±0	4.98±0	3.05±0	4.98±0	3,05±0	4.98±0	3.05±0
7	3.82±0	3.66±0.61	2.10±0.41	4.88±0	2.56±0.14	4.2±0.61	0.26±0.02	6.71±0.61
30	2.32±0.09	4.88±0	1.76±0.21	5.80±0.30	1.67±0.12	4.88±0	0.14±0	7.02±0.30
60	1.23±0.02	5.49±0.61	0.96±0.04	6.1±0	0.96±0.04	5.19±0.30	0.08±0.01	8.54±0.61
90	0.58±0.09	6.71±0.61	0.62±0	6.41±0.30	0.72±0	5.80±0.30	0	13.12±0.30

*Benzoyl Peroxide (BP) or Benzoic Acid (BA) average in grams ±SD

Table 2. Benzoyl peroxide and benzoic acid cream degradation under tested conditions

Days	Ambient		Sunlight		Refrigerator		Oven	
	BP (g)	BA (g)	BP (g)	BA (g)	BP (g)	BA (g)	BP (g)	BA (g)
0	4.98±0*	3.05±0	4.98±0	3.05±0	4.98±0	3.05±0	4.98±0	3.05±0
7	4.33±0.07	3.96±0.30	4.06±0.09	3.96±0.30	4.69±0.04	3.05±0	1.98±0	3.66±0
30	3.72±0.09	4.88±0	3.65±0.02	5.49±0	4.09±0.16	3.66±0	1.08±0.16	6.1±0
60	3.24±0.09	5.80±0.30	3.26±0.12	6.1±0	3.41±0.02	4.27±0	0.33±0.04	7.02±0.30
90	2.78±0.12	6.71±0.61	2.73±0.02	7.32±0	3.12±0.02	4.88±0	0.13±0.03	9.76±0

*Benzoyl Peroxide (BP) or Benzoic Acid (BA) average in grams ±SD

DISCUSSION

Benzoyl Peroxide, Fig [17] high reactivity is because of an oxygen bound instability. Fig [18] is the process of Benzoyl Peroxide free radicals generation. When the oxygen bound is broken, it forms benzene radicals, where propagates and can react with himself creating more benzene radicals or attack the solvent. Propagation reaction number determines chain distance force and it will only end when radicals combines their missing electrons. Solvents can accelerate the decomposition (as shown in Fig. 18 (b)) by a transfer chain process, where abenzene radical captures a solvent proton, forming a solvent radical. Benzoic Acid, Fig [19], is an example of generate product of this coming from this type of attack [4,5].

Comparing both vehicles in all tested conditions, cream is more stable to Benzoyl Peroxide degradation and Benzoic Acid formation than gel. This happens because of Benzoyl Peroxide action mechanism, where solvents radicals in cream are more stable and less reactive then benzene radicals, stopping the decomposition chain. Gel have solvents, as ethanol, who helps to accelerate kinetic decomposition chain process. Formulations with alcohol are 40% less stable when compared with acetone, propylene glycol and laureth 416. About conditions, higher temperatures facilitates the fists steps to decompose, decreasing chain distance between radicals [4].

Benzoyl Peroxide kinetic rate degradation depends on what termination is the free radical attacking. If radicals are attacking where the termination is a solvent, will have a rate equation as represented in Fig [20], and if termination is between a solvent radical and a benzoate radical, the rate equation is shown in Fig [21]. Gel Benzoyl Peroxide low R2 equation results is due to starch hydroxylation at low pH, showing a limitation in iodometric titration methodology [17]. This correspond to our results.

Researchers have been advancing to bring new formulations to Benzoyl Peroxide. Many agents were used to maintain stability as used in other formulations are infective, leading to choose encapsulated actives to drop skin irritation and at the same time retaining chemical stability, as use of hydrofluoroalkanes foams as transport nanoparticles [5]. Was tested a lipid and polymer nanoparticle incorporated in foam. The first one is more effective than the second is, because has a bigger surface tension, molecule capture and a minor particle size, showing greater efficiency comparing with simpler formulations [5]. An encapsulated Benzoyl Peroxide with polymeric micro particles to protect from degradation and avoid skin irritation [18]. Niosomal gel and a jellified emulsion prolonged Benzoyl Peroxide release, theoretically bringing more stability [19-21]. Even though, these new formulations needs to certificate product quality, so is still necessary to do stability tests as acceleration and shelf life. There is a lack of information in literature about Benzoyl Peroxide quality control of formulations and mass balance [5].

About the determination of degradation and formation products, the use of High Performance Liquid Chromatography is more common, but it is still recommended iodometric titration in European Pharmacopoeia [9] and presents same results when compared to Thin Layer Chromatography and Spectrophometric [22], being a cheaper, easier and a reliable methodology.

This article is aimed to verify manipulated Benzoyl Peroxide at 2.5% (w/w) stability in gel and cream forms, evaluating formulation at Brazilian National Formulary 2nd Edition [7] by stress test and degradation profile in 0, 7, 30, 60 and 90 days.

It is possible to conclude that Benzoyl Peroxide is an effective topic treatment for acne, but is unstable. In this article, gel samples showed a minor stability comparting to

cream, degrading more active agent and forming more reaction products in different conditions. This gel formulation is not stable because of the oxygen bond and reactions between free radicals of the drug and solvents, as alcohol, who helps to accelerate degradation. Cream formula is a better option to public sales, because of his stability and has results that are more efficient.

Development of new and more resistant formulations for Benzoyl Peroxide is necessary and challenging. Recommendation for future works is to use cream form and incorporate micelles or nanoparticles to encapsulate, slowing down degradation, maintain the cream appearance and carry the medication to release on skin. Also important to highlight to the fact that Brazilian National Formulary also the Brazilian National Pharmacopeia needs a revision to include an update on Benzoyl Peroxide formulation and analytical assays for Benzoyl Peroxide and Benzoic Acid identifications.

DECLARATION OF INTEREST STATEMENT

The authors report no declarations of interest.

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Nil

REFERENCES

1. Dias FL. Development and validation of indicator stability method of pharmaceutical formulations for topical use containing benzoyl peroxide, Universidade de São Paulo, 2014.
2. Lara VCD. Desenvolvimento de nanocápsulas contendo ácido retinóico para tratamento tópico da acne, Universidade Federal de Minas Gerais, 2008.
3. Zurita Pharmaceutical Laboratory ltda. Acnase Drug Leaflet. 2011. Available in: http://www.acneeespinhas.org/acnase-bula.htm
4. Chellquist EM, Gormam WG. Benzoyl Peroxide Solubility and Stability in Hydric Solvents. *Pharmaceut Res*., 9(10), 1992, 1341-1346.
5. Zhao Y, Brown MB, Jones SA. The Topical Delivery of Benzoyl Peroxide Using Elegant Dynamic Hydrofluoroalkane Foams. *J Pharm Sci*., 99(3), 2010, 1384-1398.
6. Agência Nacional de Vigilância Sanitária (Brasil). Cosmetic Products Stability Guide. 2004.
7. Agência Nacional de Vigilância Sanitária (Brasil). Formulário Nacional Da Farmacopéia Brasileira, 2ª edição Revisão 02:135. 2012. Available in:http://www.anvisa.gov.br/hotsite/farmacopeiabrasileira/arquivos/2012/ FNFB%202_Revisao_2_COFAR_setembro_2012_atual.pdf
8. Agência Nacional de Vigilância Sanitária (Brasil). Farmacopéia Nacional Brasileira, 5ª edição. 2010. Available in: http://www.anvisa.gov.br/hotsite/cd_farmacopeia/pdf/volume2.pdf
9. European Pharmacopoeia 8th Edition. EDQM Council of Europe, Strasbourg, France, 2013.
10. Orsoni-Segura S, Willcox N. Gel composition for treatment of common acne comprising a combination of benzoyl peroxide and adapalene and/or adapalene salt. Google patents, Galderma Research & Development, 2017.
11. Nadau-Fourcade K. Bpo wash gel composition. Google patents, Galderma S.A. 2014. Accessed in 02/16 https://www.google.com/patents/WO2014076135A1?cl=en
12. Tanarkin D, Friedman D, Schuz D, Keynan R, Berman T, Danziger J. Foam containing benzoyl peroxide. Google patents, Foamix pharmaceuticals Ltd. 2016.
13. Mallard C, Louis F, At E. Cream gels comprising at least one retinoid and benzoyl peroxide. Google patents, Galderma Research & Development. 2015. Accessed in 02/16
14. Martin B, Meunier C, Montels D, Watts O. Chemical Stability of adaptalene and tretinoin when combined with benzoyl peroxide in presence and absence of visible light and ultraviolet radiation *Br. J. Dermatol*., 139(52), 1998, 8-11.
15. Xu S, Cavera VL, Rogers MA, Huang Q, Zubovskiy K, Chikindas ML. Benzoyl Peroxide Formulated Polycarbophil/Carbopol 934P Hydrogel with Selective Antimicrobial Activity, Potentially Beneficial for Treatment and Prevention of Bacterial Vaginosis. *Infect Dis Obstet Gynecol*., 2013, Article ID 909354:10.
16. Bollinger JN, Lewis D and Mendez VM. Benzoyl Peroxide Stability in Pharmaceutical Gel Preparations. *J Pharm Sci*., 66(5), 1977, 718-722.
17. Tolvanen P, Arvela P, Sorokin AB, Salmi T, Murzin DY. Kinetics of starch oxidation using hydrogen peroxide as an environmentally friendly oxidant and an iron complex as a catalyst. *Chem Eng J*., 154(15), 2009, 52-59.
18. Carneiro J, Janzen MES, Justus B, Nadal JM, Paula JFP and Farago PV. Desenvolvimento e Avaliação de Micropartículas Poliméricas contendo Peróxido de Benzoíla. *12° Congresso Brasileiro de*, 2013.
19. Vyas J, Vyas P, Raval D and Paghdar P. Development of Topical Niosomal Gel of Benzoyl Peroxide. *ISRN Nanotecnol*., 2011, 1-6.
20. Thakur NK, Bharti P, Mahant S, Rao R. Formulation and Characterization of Benzoyl Peroxide Gellified Emulsions. *Sci Pharm*., 80, 2012, 1045–1060.
21. Gupta A, Singh S, Gkotla N, Webster TJ. Formulation and evaluation of a topical niosomal gel containing a combination of Benzoyl Peroxide and Tretinoin for antiacne activity. *Int J Nanomedicine*., 10, 2015, 171–182.

22. Daly R, Lomner LL, Chafetz L. Selective Assay of Benzoyl Peroxide in Lotions and Creams. *J Pharm Sci*., 64(12), 1975, 1999-2000.