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# International Journal of Drug Delivery Technology

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## Volume 8, Issue 2; April - June 2018

### 1. Partition Coefficient and Glutathione Penetration of Topical Antiaging: Preformulation Study

Fitria Nugrahaeni, Dewi Melani Hariyadi, Noorma Rosita

[Abstract](#)

### 2. Formulation and Evaluation of Microemulsion Based Topical Gel of Carbamazepine

Padhiar J S, Shirolkar S V, Deshkar S S

[Abstract](#)

### 3. Formulation and Evaluation of Galantamine Hydrobromide Floating Matrix Tablet

Poreddy Srikanth Reddy, Penjuri Subhash Chandra Bose, Vuppala Sruthi, Damineni Saritha

[Abstract](#)

### 4. Studies on Orally Disintegrating Tablets Prepared by Using Natural and Synthetic Superdisintegrants

Piplani Mona, Diwedi Rohini, Bhagwat Deepak Prabhakar, Pahwa Rakesh

[Abstract](#)

### 5. Preparation and Immunocharacterization of Probiotic DNA Loaded Chitosan Nanoparticles: An *In Vitro* and *In Vivo* Study

Kaur M, Bhatia A, Sethi D, Vig K, Kaur G

[Abstract](#)

### 6. Skin Penetration of Ubiquinone (Co-Q10) In Nanoemulsion Delivery System Using Virgin Coconut Oil (Vco)

Tristiana Erawati M, Widji Soeratri, Noorma Rosita

[Abstract](#)

### 7. Design and Evaluation of Ion Activated *In-Situ* Ophthalmic Gel of Brimonidine Tartarate Using Kappa Carrageenan

Vazir Ashfaq Ahmed, Divakar Goli

[Abstract](#)

### 8. Validation of Simvastatin Analysis Methods in Human Blood Plasma (*In Vitro*) Using HPLC-UV Detector

Iyan Sopyan, Cynthia Jaya, Driyanti Rahayu

[Abstract](#)

### 9. Formulation and Evaluation of Chitosan-Polyaniline Nanocomposites for Controlled Release of Anticancer Drug Doxorubicin

Dillip Kumar Behera, Kampal Mishra, Padmolochan Nayak

[Abstract](#)

10. Characterization and Release of Ibuprofen in Proniosome System (Ibuprofen-Span 60-Cholesterol)  
Tutiek Purwanti, Dewi Melani Hariyadi, Corry Silvia

Abstract

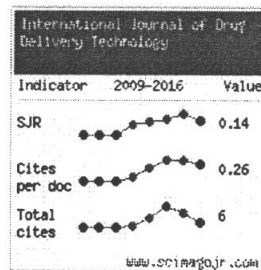
11. Evaluation of the Physical Stability of Nanostructured Lipid Carrier (NLC) Meloxicam Before and After Storage 40 Days  
Rahmi Annisa, Dewi Melani, Esti Hendradi

Abstract

12. The Optimization of Maltodextrin and Arabic Gum in the Microencapsulation of Aqueous Fraction of *Clinacanthus nutans* Using Simplex Lattice Design  
Intan Martha Cahyani, Ebita Narasukma Anggraeny, Bekti Nugraheni, Christiana Retnaningsih, V Kristina Ananingsih

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## Partition Coefficient and Glutathione Penetration of Topical Antiaging: Preformulation Study

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### ABSTRACT

Glutathione (GSH) is a broad antioxidant of the thiol-tripeptide group, highly hydrophilic, which has limitation for topical preparations. A lipophilic surfactant is an alternative method to enhance the glutathione partition. The purpose of this study was to determine the apparent partition coefficient (APC log) of glutathione; glutathione with additional surfactant at different HLB value of HLB 4.3; 5.5; 7; 11 and selected HLB was studied for penetration. The study was conducted by dissolving glutathione in water plus various HLB surfactants. Determination method of partition coefficient was done by shake flash method. The penetration test was conducted using the parameter of decreasing Matrix Metalloproteinase-1 expression on the balb-c male skin. The results can be used as a reference for topical glutathione formulations as these results are preformulation study.

**Keywords:** glutathione, coefficient of partition, HLB value, surfactant, penetration, matrix metalloproteinase-1.

### INTRODUCTION

Disadvantages of UV radiation, either directly or indirectly. UV damage which is estimated to occur about 50% due to free radical formation. As a result of this condition fundamental structure will decrease the amount of collagen and increase the level of the metalloproteinase-1 matrix (MMP-1)<sup>1</sup>. One of the antioxidants in the body that can be used to overcome the problem of protein-tyrosine phosphatase reduction is L- $\gamma$ -glutamyl-L-cysteinyl-glycine (glutathione).

Glutathione in the body is in reduced form. Glutathione is antioxidant that has a SH group on the amino acid cysteine. Glutathione concentrations that decline with age lead to the symptoms of aging. Glutathione can also function as a depigmentation agent. Glutathione is an important cofactor for many enzymes and is involved in several metabolic and signal pathways. glutathione is also important for the regeneration of other antioxidants such as tocopherol and ascorbate<sup>2</sup>.

Glutathione has low oral bioavailability due to low absorption in the gastrointestinal tract due to the action of the  $\gamma$ -glutamyl transpeptidase (GGT) enzyme that decreases glutathione<sup>3</sup>. Potential side effects occur when administration of intravenous glutathione in the blood circulation. One attempt to avoid a first pass effect is to use a topical route<sup>4</sup>.

Besides, since glutathione has a log P value of -1.4 and hydrophilic, it is necessary to increase the lipophilicity of glutathione to be used topically. Excess topical use includes local effects, and minimal systemic side effects. Besides the problem of lipophilicity, another glutathione problem is stability, especially in neutral or alkaline environments<sup>5</sup>.

Glutathione as an antioxidant will work on the epidermal layer and dermis<sup>6</sup>. Based on research that glutathione is applied topically to the skin by using an oil emulsion with plain water or the form of conventional gel is not able to penetrate through stratum corneum, so it is not useful in inhibiting UVB<sup>7</sup>. Stratum corneum skin is the primary penetrating barrier for all substances, consisting of a horn layer. The horn layer is part of the epidermal layer is selective in selecting specific compounds to enter the broader layers or dermis layer, so not all active facial ingredients have compounds capable of penetrating the coating<sup>8</sup>. Antiaging is products such as glutathione work in the dermis layer. If glutathione can pass through intercellular and transcellular paths, it can increase the effectiveness of topical glutathione use. One of the factors that can affect penetration ability through intercellular and transcellular lines is the value of the partition coefficient (log P). The optimal P log value for penetrating the substance through the stratum corneum is 2-3<sup>9</sup>. Glutathione has a low penetration ability in the skin, to takes glutathione to pass through the stratum corneum to the dermis layer by increasing lipophilicity of glutathione. One method to improve lipophilicity of drugs using surfactants<sup>10</sup>. So far, lipophilic drugs have lowered lipophilicity by the addition of surfactants. Indomethacin with tween 80 and sodium lauryl sulfate (SLS) experienced significant increases in dissolution. Megestrol acetate can be improved solubility and bioavailability using the newly synthesized surfactant Rofam 70 and rapeseed methyl ester ethoxylate<sup>11</sup>. Drug formulations that can increase permeation rate across the membrane are affected by physical conditions and nonionic surfactant concentrations<sup>12</sup>.

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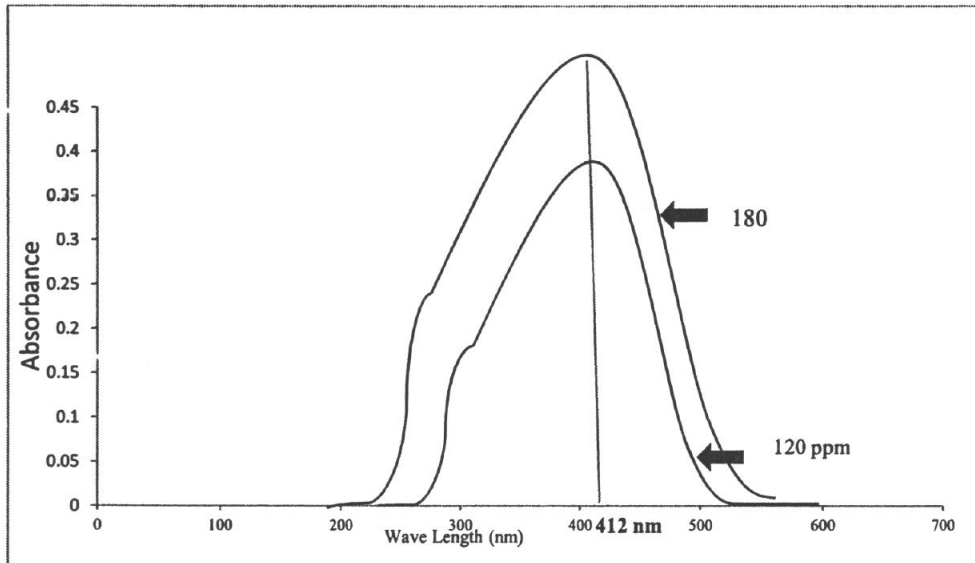


Figure 1: Absorption Profile of Glutathione Standard Solution 120 ppm, and 180 ppm in 0.01 M phosphate buffer solution pH 6.0 ± 0.05.

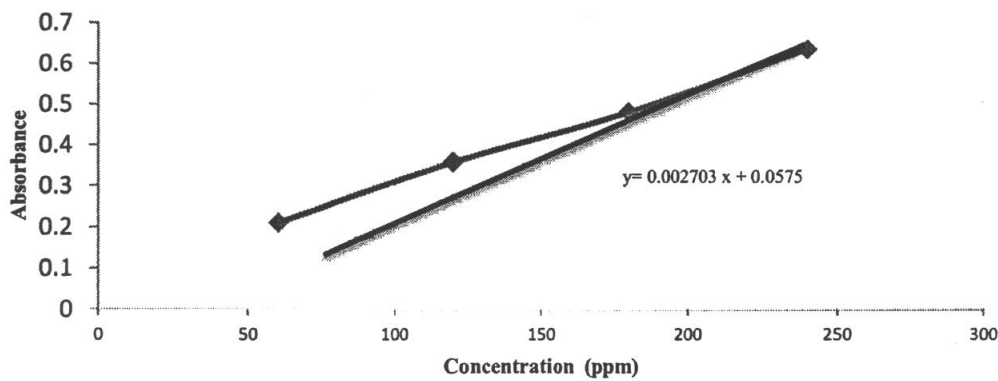


Figure 2: Glutathione in Phosphate buffer pH 6.00 ± 0.05.

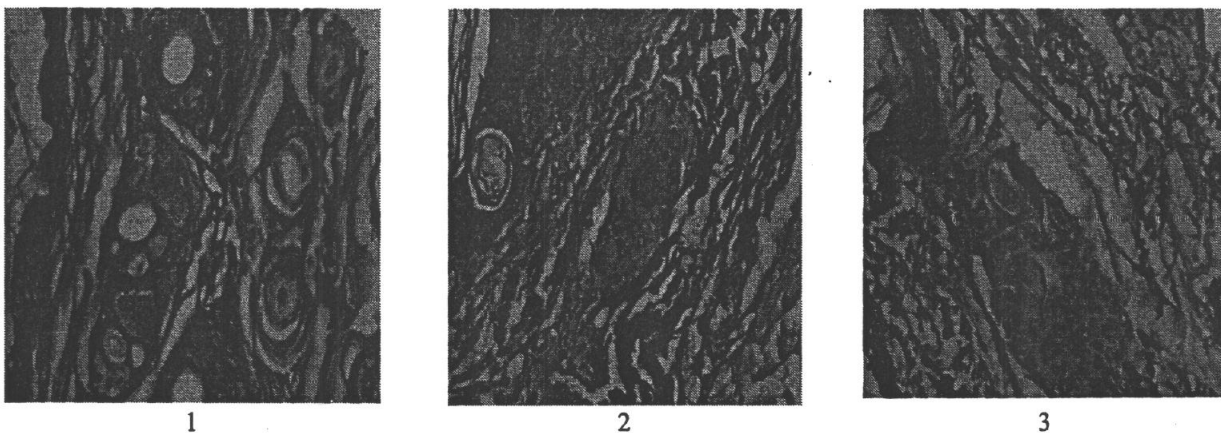


Figure 3: MMP-1 Expression of Dermis Mice with Immunohistochemical Painting (1) gel glutathione with surfactant, (2) gel glutathione without surfactant, (3) gel base (Magnification 400x).

In this study lipophilicity of glutathione was attempted to be increased by the addition of surfactant. The selected surfactant is nonionic with various levels of Hydrophile-Lipophile Balance (HLB). Nonionic surfactants are chosen for their excellent percutaneous tolerance, less irritation and toxicity potential<sup>13</sup>. The HLB value shows the strength of hydrophilic and lipophilic properties of the surfactant. The lipophilic surfactants will have low HLB values and

are more soluble in oil, while hydrophilic surfactants have high HLB values and will be more water solvent<sup>14</sup>. Therefore it needs to be tested its effect on glutathione partition coefficient. The enhanced glutathione lipophilicity with the surfactant is then determined by its penetration using effectiveness test parameters based on the decrease in MMP-1 levels.

Table 1: Glutathione and Partition Coefficients in phosphate buffer pH 6.0 ± 0.05 (water phase) at the fifth hour temperature 37 ± 0,5 ° C.

Glutathione +HLB Value	Average Log APC ± SD	%KV
4,3	1.535±0.004	0.260
5,5	1.632±0.032	1.960
7	2.259±0.009	0.398
11	1.750±0.016	0.914
13	1.300±0.012	0.923
GSH Without surfactant	1.224±0.006	0.490

Table 2: Results of Expression MMP-1.

Formula	Average Expression: MMP-1 (%) ± SD
GSH+Surf	24,25 ± 8,79
GSH - surf	63,49 ± 0,60
control	72,03 ± 0,59

## MATERIAL AND METHODS

### Materials

Materials: Reduced L-Glutation- ≥ 98.0% (Sigma Aldrich), Tween 80, Span 80, 5,5-dithio-bis- (2-nitrobenzoic acid) (DTNB) Ellman's Reagent (Sigma Aldrich), Kit and Antibodies Matrix Metalloproteinase- 1, ethanol pa (Merck), Mus mucus male balb-c strain.

### Enhancement of Lipophilicity

Glutathione 2 grams was dissolved in 20 ml phosphate buffer solution pH 6 ± 0.05 then surfactant as added until reach HLB value of 13;11;7; 5.5;4.3 with the addition of Tween 80 and Span 80 of 0.5 g. Then it was freeze dried -26°C for 30 hours.

### Determination of Wavelength Maximum (λmax) Glutathione

The maximum wavelength determination was done by observing the absorption value of 4 levels of standard glutathione solution at 200-500 nm wavelength.

### Determination of Partition Coefficients Apparent Glutathione and surfactant

Determination of apparent partition coefficients was carried out in phosphate buffer solution pH 6.0 ± 0.05 saturated octanol and a saturated phosphate buffer phosphate buffer solution of pH 6.00 ± 0.05. The initial glutathione content in the aqueous phase used was 200 µg / mL (20 mg ad 100 ml). The phase water volume used for the determination of the partition coefficient is 10.0 mL. The solution was introduced into the vial and added 0.50 ml of a saturated organic solvent (n-octanol) with a phosphate buffer solution of pH 6.0 ± 0.05. The mixture between a water phase containing glutathione and a saturated n-octanol is shaken with thermoshaker until the equilibrium time is reached at 37 ° ± 0.5 ° C with a frequency of stirring of 150 rpm. After that. The absorption was examined using a UV-Vis spectrophotometer at a maximum wavelength to determine the glutathione content of the water phase. The determination of the apparent coefficient is performed by replicating three times. The value of the apparent partition coefficient can be calculated

after the glutathione concentration is obtained in the water phase after equilibrium using the following equation:

$$APC = \frac{(c_2^0 - c_2^1).a}{c_2^1.b}$$

Notes:

APC: apparent partition coefficient

$C_2^1$  : the concentration of the drug in the water phase after the equilibrium occurs

$C_2^0$  : the concentration of the drug in the water phase before the equilibrium occurs

a: water phase volume

b: volume of an oil phase

### Glutathione Effectiveness Test Based on Decreased MMP-1 Expression

Thirty balb / c mice were first adapted for seven days. UV irradiation is performed every 2 days, ie on days 1,3,5,7,9,11 and 13 (Mice are left for twenty-four hours after irradiation ends to exclude the effects of acute irradiation effect with doses of each irradiation 60mJ/m<sup>2</sup>. The first group was applied to the glutathione gel glucose group HLB 7. The second group was applied gel glutathione The third group was the gel base group.

## RESULTS

*Determination of the maximum wavelength of glutathione in 0.01 M phosphate buffer solution pH 6.0 ± 0.05.* Based on the result of a determination of maximum wavelength obtained is 412 nm. Profile of glutathione spectra in 0.01 M phosphate buffer pH 6,0 ± 0.05 can be seen in figure 1.

### Determination of Standard Curve

The determination of standard curve was done by observing the absorption of various glutathione concentrations in 0.01 M phosphate buffer pH 6,0 ± 0.05 at a wavelength of 412 nm. The results can be seen in figure 2. From the standard curve obtained linear regression equation  $y = 0,002703 x + 0,0575$  with correlation coefficient (r) = 0.99905.

### Determination of Coefficient of Apparent GSH of Freeze Dried Samples

The determination of the apparent coefficient is performed as in the equilibrium timing; the selected equilibrium time is at fifth hour, the sample is taken by replication three times. The value of the apparent partition coefficient can be estimated using equation. Acquisition of glutathione levels in the water phase or buffer solution and APC log value can be seen in table 1.

The coefficient of glutathione partition with the addition of various surfactants of various HLBs approaching 2-3 according to the Log P skin is glutathione with the addition of HLB 7 surfactant which is a mixture of Span 80 of 2.52 ml with Tween 80 of 7.47 ml. Furthermore, glutathione processed by microspheres is glutathione with the addition of HLB 7 surfactant (Glutathione with surfactant).

### Glutathione Penetration Based on Effectiveness Test Result on MMP-1 Expression Decrease

In MMP1-1 expression test results obtained the average MMP-1 expression results presented in table 2.

Based on statistical analysis of variance (ANOVA) on MMP-1 expression test, p-value (sig) 0.000 less than 0,050 is obtained. It is suggested that there is a significant

Table 3: Results Anova Expression MMP1.

ANOVA					
MMP1	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7418.927	4	1854.732	72.954	.000
Within Groups	254.232	18	25.423		
Total	7673.159	14			

difference in MMP-1 expression in dermis tissue due to differences in formulas. After the Post Hoc analysis was conducted to determine which groups were different, it was found that there was a difference between the treated group of glutathione with surfactant gel and glutathione without surfactant gel, and a control group.

## DISCUSSION

From the research conducted, the maximum wavelength of glutathione in 0.01 M phosphate buffer pH 6,0 ± 0.05 is 412 nm. Observation of absorbance of glutathione is carried out at its maximum wavelength because the absorbance change for each one unit is the largest at the maximum wavelength, so that maximal sensitivity analysis can be obtained<sup>15</sup>. While the linear regression equation obtained from the standard curve is  $y = 0.002703x + 0.0575$  with the correlation coefficient ( $r$ ) = 0.99905. The correlation coefficient value obtained is greater than  $R_{table} = 0.9911$  at a significant level of 0.1% indicating a confidence level of 99.9%. It shows there is a linear relationship between the concentration of a standard solution and the absorbance.

The equilibrium time is the time required for a substance to achieve its saturation state, which is necessary as a basis for determining the solubility time of a drug substance. In this study, the equilibrium timing was performed by using phosphate buffer pH 6.0 ± 0.05 saturated octanol at 37 ± 0,5°C with sampling every hour for seven hours starting from the third hour. After the calculation of the level can be known when the equilibrium glutathione is at the fifth hour. This is evidenced by the value of % KV of 1.04 for glutathione levels at the fifth, sixth, and seventh hours.

The partition coefficient is the ratio of the solubility of a drug in the oil phase and the water phase, which can be used to predict the large number of drugs that can penetrate the skin membrane to reach the receptor<sup>16</sup>. In this case, the body skin membrane can be identified with the oil phase, and the body receptor is the water phase.

Before the determination of the partition coefficient, the first saturation phase of n-octanol and 0.01 M phosphate buffer pH 6,0 ± 0.05 for one day and one night. Water phase used in this research is phosphate buffer solution pH 6,0 ± 0,05. The oil phase used is n-octanol. The saturation of these two steps is intended to ensure that the results of the partition coefficients obtained are accurate. The ratio of n-octanol and buffer in each phase was in a constant state so that the amount of dissolved drug in each stage did not change significantly.

The determination of glutathione partition coefficient was carried out at 37 ± 0,5 ° C, i.e., at the fifth hour by using shake flask method. Samples were taken by replication three times when the equilibrium time was reached and observed their absorbance by UV-Vis spectrophotometry.

From the absorbance of samples obtained can be calculated glutathione levels are still left behind. After the obtained levels of glutathione in the water phase after going equilibrium, it can be calculated the value of apparent partition coefficient. From the value of the apparent partition coefficient obtained, it can be calculated the average value of P oct/buffer phosphate log, HLB 4.3 of 1.535 ± 0.004 (% KV = 0.26%), HLB 5.5 average P oct/buffer phosphate Logs of 1.632 ± 0.032 (Mean KV = 1,96), HLB 7 average P oct/buffer phosphate log of 2.259 ± 0.009 (% KV = 0.398), HLB 11 Average P oct/buffer phosphate log of 1.750 ± 0.016 (% KV = 0.914), HLB 13 averaged Log P oct/ buffer phosphate of 1.300 ± 0.012 (% KV = 0.923), GSH without an average surfactant Log P oct/ buffer phosphate of 1.224 ± 0.006 (% KV = 0.490). The results are by the principle of like dissolve like, polar compounds soluble in polar solvents, as well as any non-polar ones soluble in non-polar solvents. When the glutathione solution is mixed with HLB surfactant below 7, there is the separation between the oil phase and the water phase after ethanol is completely evaporated. The partition coefficient value drug to penetrate the stratum corneum between 2-3 indicates optimal skin penetration<sup>17</sup>. The results of the above study which between the log P 2-3 is HLB 7 so it can be seen that glutathione has lipophilic and hydrophilic properties sufficient to penetrate the stratum corneum well.

The selected glutathione was then fed into the gel base for effectiveness test seen from decreasing MMP-1 levels in mouse skin with UV irradiation every two days with a dosage of each irradiation of 60mJ /m<sup>2</sup>. The mean of MMP-1 expression was obtained in control group with only gel base (control) of 72,03%; gel glutathione with surfactant group was 24.25%, a group of glutathione without surfactant was 63.49%. After one way ANOVA test and post hoc test between control group and gel glutathione gel group were significantly different. The gel glutathione without surfactant group was substantially diverse from the glutathione with surfactant gel group. It indicated that glutathione with surfactant can decrease MMP-1 expression in mice dermis tissue. It is because glutathione has increased lipophilicity approaching log P 2-3 so that it penetrates through the stratum corneum into the dermis tissue.

Increased MMP-1 expression after radiation on the skin of the mice group for two weeks. It is because the energy from UV radiation damages cell membranes and proteins to produce reactive oxygen species (ROS), which induce expression of proinflammatory cytokines binding to cell surface receptors including receptors of epidermal growth factor, interleukin (IL) -1, insulin keratinocyte growth factor and tumor necrosis factor (TNF)<sup>18</sup>. Activation of the three receptors will activate the MAPKS (Mitogen-Activated Protein Kinases) intracellular signals. Activation of the kinase will induce the transition of the AP-1 core complex. Transcription of the MMP-1 gene will increase and will decrease procollagen 1 and 3, and decrease the TGF-β receptor so that the dermal matrix formation will fall. In the skin, the action combination of collagenase (MMP-1), gelatinase (MMP-2, MMP-9), and



stromelysin-1 (MMP-3) can degrade collagen and components of the elastic tissue. UV B rays with low dose exposure can cause redness of the skin (erythema), inducing regular and regularly regulated MMP-1, MMP-3 and MMP-9 expression<sup>19</sup>.

## CONCLUSION

Based on the results of this study it can be concluded that glutathione with surfactant has a log P of 2.23. Glutathione with surfactant penetration test results can decreased MMP-1 expression therefore it is recommended to use efficiently as a topical agent.

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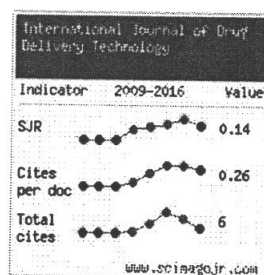
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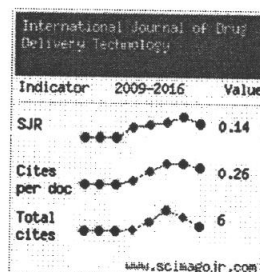
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H Index

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Pharmaceutical Science	2012	Q4
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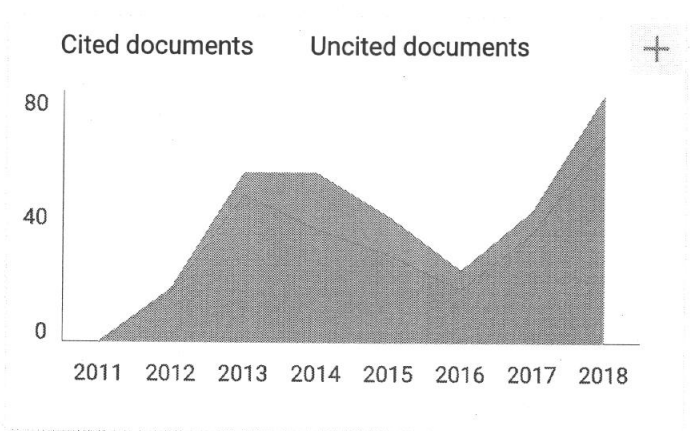
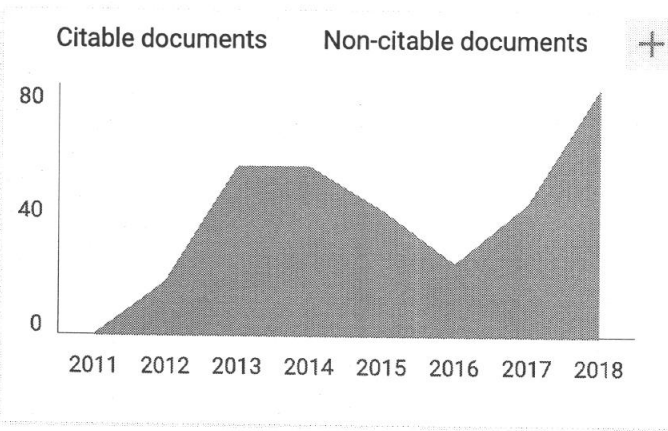
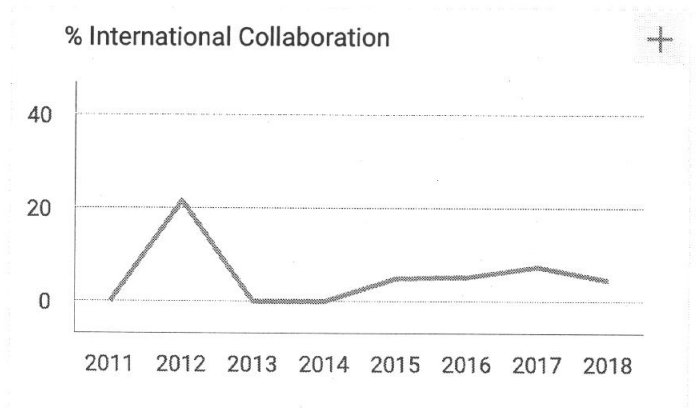
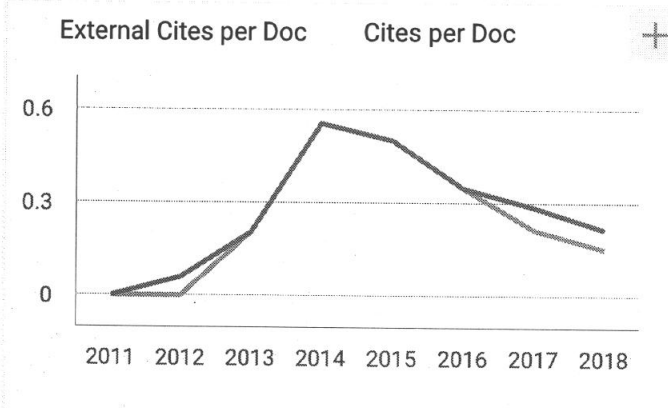
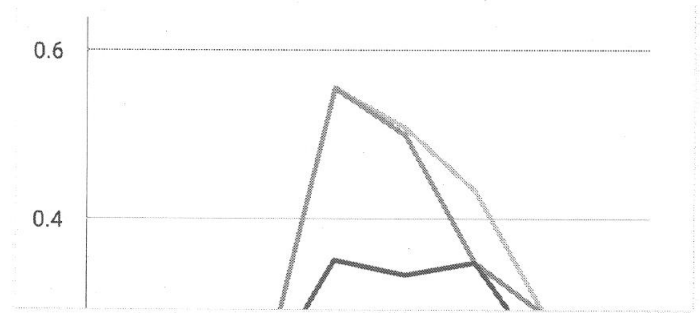
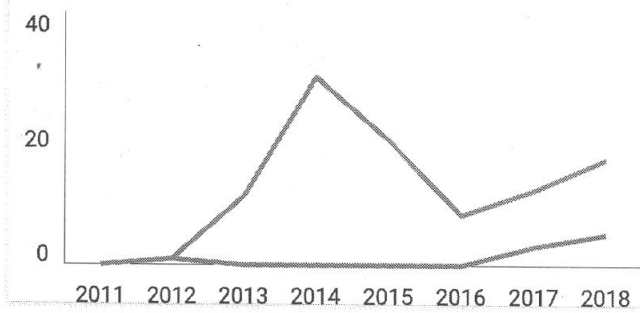
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