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Original Research Article

Reactive Separation of Gallic Acid Using Phosphoric and Aminic Extractants in Non-Toxic Natural Diluents

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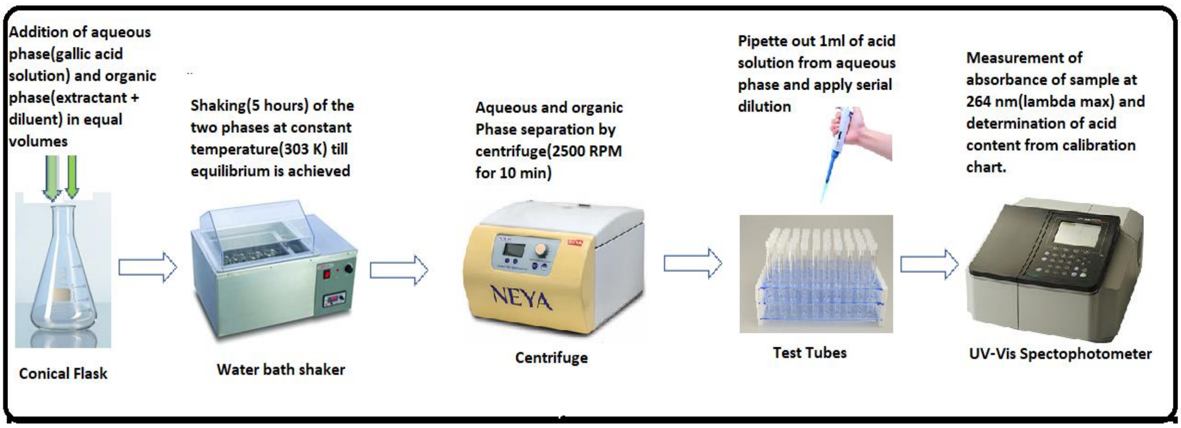
ABSTRACT

Bio production of gallic acid and its recovery from bioconversion media have captured considerable interest of researchers. Extractive separation with solvents can be utilized for the recovery of gallic acid; however, the issue of toxicity of solvents towards microbes needs to be addressed. Physical extraction with natural solvents is observed to be insufficient as highest distribution coefficient (K_D) were observed to be 0.136 for sesame oil, 0.111 for rice bran oil and around 0.041 for sunflower oil. Higher separation efficiencies are desirable for a successful extraction system and in this regard employing a reactive component (extractants) could be intensified solution. Three different extractants (tri-n-butyl phosphate (TBP), tri-octyl amine (TOA) and Aliquat 336) were investigated for their capabilities to improve extraction when present in three non-toxic diluents (sunflower oil, rice bran oil and sesame oil) comprising 9 possible combinations employed over acid concentrations (0.01-0.05 kmol.m^{-3}). TBP-sunflower oil (K_D = 0.81-15.66, K_E = 1.922 - 7.894), Aliquat 336-rice bran oil (K_D = 3-10.71, K_E = 6.378-10.67), and TOA-sesame oil (K_D =0.1-3.54, K_E = 2.081-3.409) combinations were observed to result in most suited systems resulting in higher distribution of acid. Thus, they were found to be ideal for efficient and low toxic extractant systems for acid recovery.

GRAPHICAL ABSTRACT



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Introduction

Gallic acid (3,4,5 trihydroxy benzoic acid) is commercially used in pharmaceutical industries owing to its medicinal properties like antioxidant, antimutagenic and anti-inflammatory [1]. It is present in minute quantities in free form in the naturally occurring sources like gall nuts, tea leaves, oak bark and is usually produced commercially by hydrolysis of tannins. Commercial methods produce toxic compounds as by-products, and environmentally friendly methods of production are being researched. One of these methods is the bioconversion of tannins to gallic acid by naturally produced enzyme tannase by various microbes [2-5]. Among the various categories of microbes, *Aspergillus niger* are more effective producers of the enzyme tannase which helps in the breakdown of the bigger tannin molecule and leads toward the production of gallic acid. The downstream refining involves separation the acid from the bioconversion broth and its recovery in pure form. Some techniques for recovery of organic acid from the fermentation broth are classical precipitation method, chromatography, membrane separation methods and liquid-liquid extraction [6]. Except the precipitation method which produces lots of waste and slurry solid, the other methods are in the developmental stage [7-10].

Liquid-liquid extraction offers economical way to separate carboxylic acid from complex fermentation media but suffers from the disadvantage that there is low distribution of acid in the organic phase. Reactive extraction, involving the use of extractants, can be employed to enhance the distribution of acid in the organic phase and has been researched by many researchers for the recovery of carboxylic acids from the dilute aqueous streams or fermentation broth [11-16]. The production of acid lowers the pH in the broth and impairs the growth of microbes in the media; therefore, researchers have proposed reactive extraction based in situ product removal technology (ISPR) [17]. However, the toxicity of diluents and extractants to microbes is an issue that needs to be addressed as most of the diluents are toxic to microbes. This can be avoided by using indirect contact technique that involves the use of membrane contactors or by cell immobilization. A simple alternative to these expensive technologies could be the development of biocompatible solvent mixture that can be non-toxic or less toxic towards microbes. Diluent toxicity is a function of log*P* (where log*P* is the logarithm of the distribution coefficient (KD) of the diluent in a standard octanol-water system) as elaborated by some authors [18-19]. The diluents with log*P* lower than 4 are considered to be toxic whereas they are considered to be non-toxic if log*P* is above than 6. A biocompatible and efficient mixture can be obtained by blending of toxic extractants with some non-toxic natural diluents such as sunflower oil, rice bran oil and sesame oil, without compromising to the recovery yield. Direct contact reactive extraction with the proper selection of diluents-plus-extractants and suitable resistant microorganisms, can provide high selectivity and extraction, leading towards efficiency in situ product removal.

There are a few studies for the reactive extraction of carboxylic acids with the natural diluents such as sunflower oil, rice bran oil, sesame oil, canola oil and soybean oil [20-28].

Although there are some equilibrium studies for the reactive separation of gallic acid [29-38] and other phenolic acid such as benzoic acid [39] and protocatechuic acid [40-42], equilibrium studies with non-toxic natural diluents for the gallic acid separation have never been reported. Therefore, in the present work the different combination of non-toxic natural diluents such as sunflower oil, rice bran oil and sesame oil with different extractants such as tributyl phosphate, tri octyl amine and Aliquat 336 for the physical and reactive extraction process has been presented.

Theoretical

For the concentration of acid solutions such that pH is less than the pK_a values of acid, the acid transfers into organic solvent as presented by Kertes and Kings [43].

Overall distribution coefficient (K_D) is defined as the ratio of total concentration of acid in its all forms in organic phase and total concentration of acid in aqueous phase. For physical extraction, K_D

is expressed in terms of partition coefficient(P) and dimerization coefficient (D) as Equation 1:

$$K_D = \frac{P+2P^2D[HA]_{aq}}{1+ {K_{HA}}/{[H^+]_{aq}}} \quad 1$$

The reactive extraction equilibrium of carboxylic acid (HA) using different extractant (E) can be represented by Equation 2, showing acid: Extractant complex formation at the interphase.

$$\mathrm{HA} + s\mathrm{E}_{\mathrm{org}} \leftrightarrow \mathrm{HA}\cdot (\mathrm{E})_{s,\mathrm{org}} \quad 2$$

Using mass action law, a relationship as presented in Equation 3 can be presented to evaluate the values of complexation constant (K_E) and solvation number (s) for the Equation 2 from experimentally observed quantities.

$$\log K_{\mathrm{D}} + \log(1 + K_{\mathrm{HA}}/ [H^+]) = \log K_{\mathrm{E}} + s \log [\mathrm{E}]_{\mathrm{org}} \quad 3$$

The equilibrium extractant concentration can be determined by subtracting complexed extractant from initial extractant as given in Equation 4.

$$[\mathrm{E}]_{\mathrm{org}} = [\mathrm{E}]_0 - [s^*\mathrm{HA}.\ \mathrm{E}_s] \quad 4$$

For initial concentrations much higher than complexed extractant we can neglect the later and take $[\mathrm{E}]_{\mathrm{org}} = [\mathrm{E}]_0$.

The extraction efficiency (E %) can be obtained from the Equation 5.

$$E = \frac{K_D}{1+K_D} * 100 \quad 5$$

Loading ratio(Z) of the extractant, defined as total concentration of acid in the organic phase to extractant concentration is expressed as

$$Z = \frac{[\mathrm{HA}]_{\mathrm{org}}}{[E]} \quad 6$$

Material and methods

Sunflower and sesame oil were procured from Nature Land Organic Foods Pvt Ltd; India and rice bran oil was procured from RV Essential, India. Table 1 lists out the makeup, molecular formula and purity of chemicals used in the work. The solvents were used directly without further purification. Ultrapure water (obtained from Millipore water purifier) was used for solution preparation. The chosen operating conditions for the experiments included temperature = 303 K and pressure =101.325 kPa. Gallic acid solutions were prepared in the concentration range of 0.01−0.05 kmol.m^{-3} as found in bioconversion media and olive mill wastewater.

The experiments involved shaking 10 ml acid solution (concentration= 0.01-0.05 kmol.m^{-3}) with 10 ml of organic phase composed of diluents (rice bran, sunflower and sesame oil) with extractant (TBP, TOA, Aliquat 336), respectively, for 5 hours at fixed temperature (T=303 K). This time, it was calculated based on preliminary experiments and was sufficient to reach equilibrium. After shaking, the phases were separated by centrifuging at 2500 RPM for 10 minutes to get clear separation. The aqueous phase was then analyzed by UV spectrophotometer (Shimadzu1800, Japan) at wavelength of 264 nm for the determination of acid present in it. The organic phase acid concentration was calculated by mass balance under the assumption that there was negligible water for extraction. Also, it was assumed that the solubility of tributyl phosphate, tri-n-octyl amine and Aliquat 336 in water was negligible. The pH of the acid in aqueous phase initially and at equilibrium was recorded and it was observed that during the whole experiment the pH remained less than pK_a (4.40) of the acid. This condition (pH<pK_a) is highly desirable as its fulfilment leads to very low dissociation of acid in the aqueous phase.

Table 1: Chemicals used in the present work

Chemical	Supplier	Molecular formula	Purity (%)
Gallic Acid	Molychem, India	$C_7H_6O_5.H_2O$	99.5
Tributyl phosphate (TBP)	Loba Chemie Pvt. Ltd, India	$C_{12}H_{27}O_4P$	99
Tri-n-octylamine (TOA)	SRL Pvt. Ltd., India	$C_{24}H_{51}N$	95
Aliquat336 (A336)	Loba Chemie Pvt. Ltd. India	$C_{25}H_{54}NCl$	88

Results and Discussions

Physical extraction with only the natural solvents sunflower, sesame and rice bran oil was studied and it was observed that the extraction was negligible in all the cases as reflected by the poor distribution coefficient and extraction efficiency. The physical extraction results are presented in the form of equilibrium curves where $\mathrm{HA}_{\mathrm{org}}$ is plotted against $\mathrm{HA}_{\mathrm{aq}}$ as shown in Figure 1.

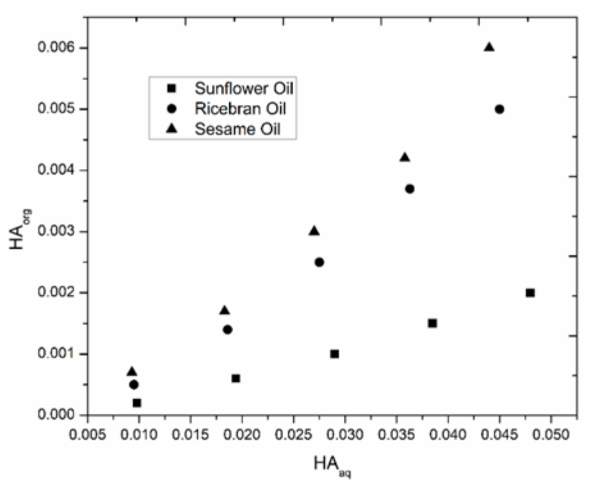

Figure 1: Physical Extraction equilibrium curves for the separation of gallic acid using non-toxic diluents (sunflower oil, rice bran oil and sesame oil)

The distribution coefficient as well as the extraction efficiencies for all the diluents are quite low and this suggests that physical extraction is not an effective option. Dimerization (*D*) and partition coefficient (*P*) were calculated for sunflower oil (*P*= 0.018, *D*=817.28), rice bran oil (*P*= 0.042, *D*=459.66), and sesame oil (*P*= 0.061, *D*= 224.44) from the Equation 1, as shown in Figure 2.

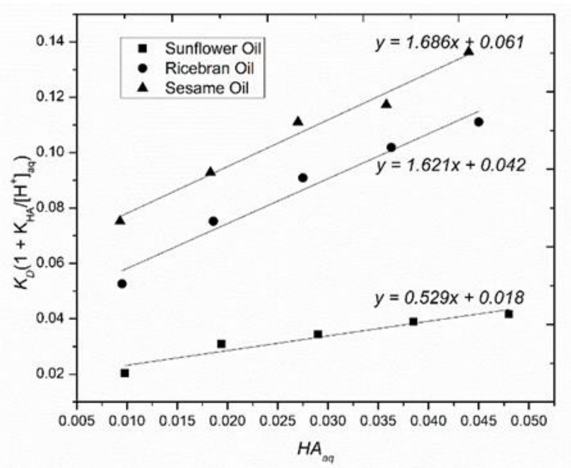

Figure 2: Plot for the determination of *P* and *D* values for various diluents

Results clearly indicate that there is low partitioning of acid as represented by the lower values of partition coefficient (*P*). Also, the higher dimerization coefficient values indicate that there is high tendency of gallic acid molecules to occur in dimeric form in the organic phase. These two coefficients partition and dimerization coefficient were observed to show inverse relation for the three natural solvents, i.e., the higher the partition coefficient, the lower is the dimerization coefficient.

Chemical extraction was studied using three different types of extractants (TBP, TOA and Aliquat336) in the volume percent of 10 to 40% (v/v) in the diluents employed. The extractants were not used above the 40 % v/v in various diluents as high excess of extractants could lead to toxicity problems. Although the phenomenon of extraction for all the three extractant is reversible complexation, the mechanism of the phosphorus and aminic extractants is quite different. Phosphorus bonded oxygen bearing extractant (TBP) extracts the acid by the solvation mechanism through the formation of hydrogen bond. The mechanism of extraction by amines is the formation of ion pair. Extractants such as quaternary ammonium salt (Aliquat336) can extract both dissociated as well as undissociated form of acid but are very viscous thus unable to provide very high distribution coefficients.

It was observed that with the addition of extractant (tributyl phosphate, tri-n-octyl amine and Aliquat336), the separation efficiency (*E* %) increased significantly. This increase in separation efficiency clearly suggests that chemical extraction is better than physical one. The effect of diluent type, extractant type and concentration and effect of initial acid concentration have been discussed further with nature of complexation and loading explained in depth.

Effect of Diluents

In the studies involving TBP as extractant, the three natural diluents were performed differently. It can be observed that combination of TBP and sunflower oil provides highest distribution coefficient (K_D in the range of 0.81-15.66), followed by TBP + rice bran oil (K_D = 0.77 to 13.43), followed in turn by TBP in sesame oil (K_D = 0.11 to 11.5). The variation of average distribution coefficient (K_D) for increasing volume percentage for TBP for the three diluents is shown in Figure 3. Though there is continuous increase of average distribution coefficient as the volume % of TBP is increased, the highest jump in the average distribution coefficient is observed from 30 volume% to 40 vol % of TBP in all the oils.

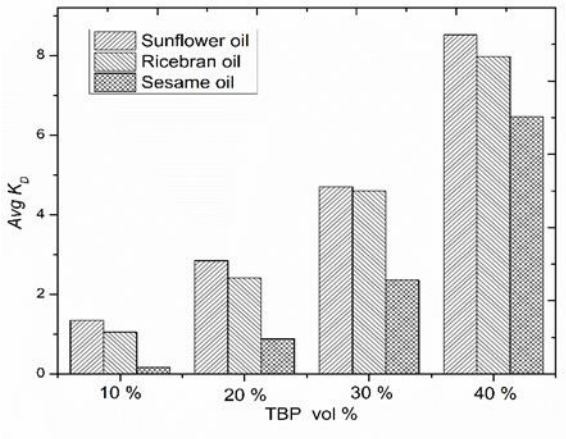

Figure 3: Variation of average distribution coefficient (Avg K_D) with TBP volume percentage in sunflower oil, rice bran oil and sesame oil

Among the various TOA diluent combinations, TOA + sesame oil provided higher distribution coefficients (KD in the range of 0.10-3.54) followed by TOA + rice bran oil (K_D = 0.17 – 2.44) and TOA + sunflower oil (K_D = 0.07 – 2.33). The similar trend is shown by the variation of average distribution coefficient for various TOA volume percentages for the three oils as presented in Figure 4. The highest increase in the average distribution coefficient was observed in the sesame oil when TOA was varied from 30 to 40 % by volume.

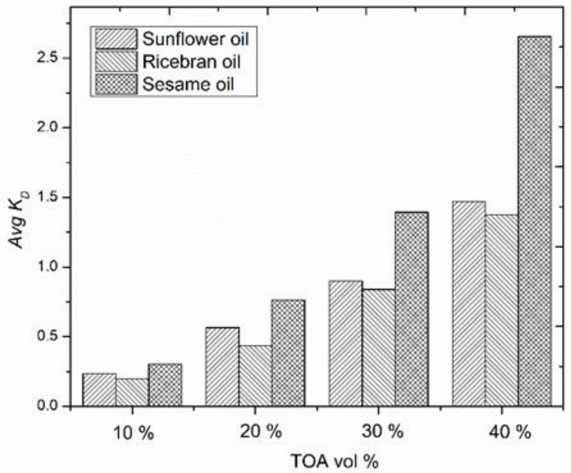

Figure 4: Variation of average distribution coefficient (*Avg K_D*) with TBP volume percentage in sunflower oil, rice bran oil and sesame oil

The variation is of average distribution coefficient obtained when different volume % of Aliquat336 was employed in the three natural diluents as depicted in Figure 5. Aliquat 336 +rice bran oil provided the higher distribution coefficients (in the range of 3-10.71) followed by sesame oil (K_D in the range of 1.77-10.11) and sunflower oil (K_D in the range of 0.25-7.89).

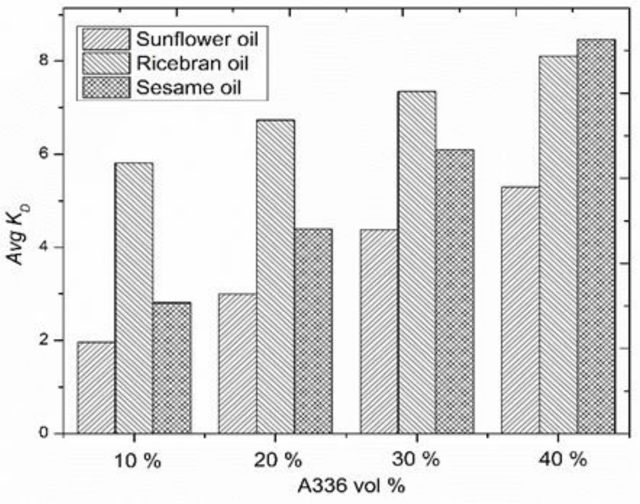

Figure 5: Variation of average distribution coefficient (*Avg K_D*) with aliquat336 (A336) volume % in sunflower, rice bran and sesame oil

Effect of Extractant Concentration

In the three diluents, i.e., rice bran, sesame and sunflower oil, each extractant behaved differently. In sesame oil, the extractant Aliquat 336 best provided higher distribution coefficient except at the highest initial acid concentration by 10.11 lower that that provided by TBP (K_D = 11.5). The extractant TOA provided distribution coefficients in the range of 0.10-3.54 lower than both A336 and TBP. In the sunflower oil, the highest distribution coefficients were provided by TBP (in the range of 0.81-15.66) followed by that of Aliquat 336 (providing the distribution coefficient in the range of 0.25 – 7.89) and TOA (providing K_D in the range of 0.07 -2.33). In the rice bran oil, although the extractant A336 provided the higher average distribution coefficient, the highest distribution coefficient was provided by TBP (K_D = 13.43) at the highest initial acid concentration of 0.05 kmol.m-1. TOA again did not act well providing distribution coefficient in the range of 0.17-2.44. The variation of average distribution coefficient (*Avg K_D*) for various extractants (TBP, TOA and A336) in sunflower oil, rice bran oil and sesame oil is shown in Figures 6, 7 and 8, respectively.

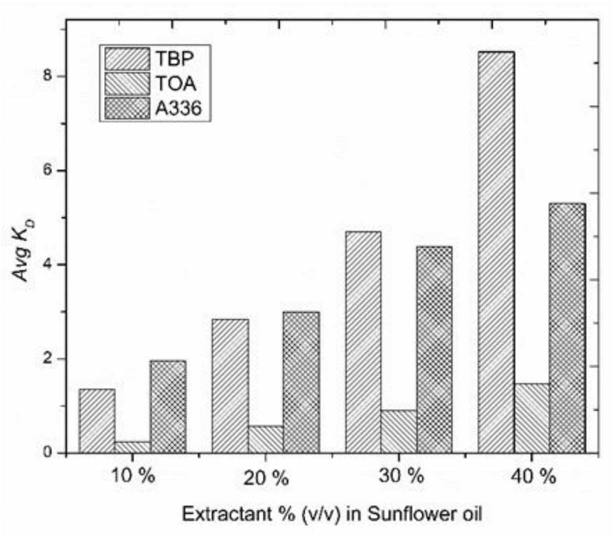

Figure 6: Variation of average distribution coefficient (*Avg K_D*) for different extractants in sunflower oil

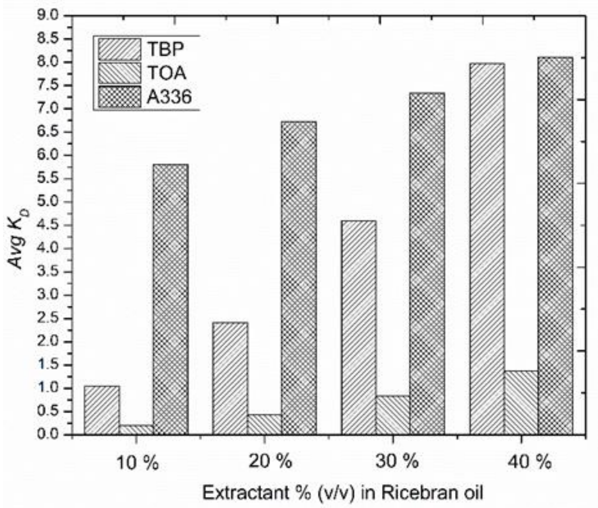

Figure 7: Variation of average distribution coefficient (*Avg K_D*) for different extractants in rice bran oil

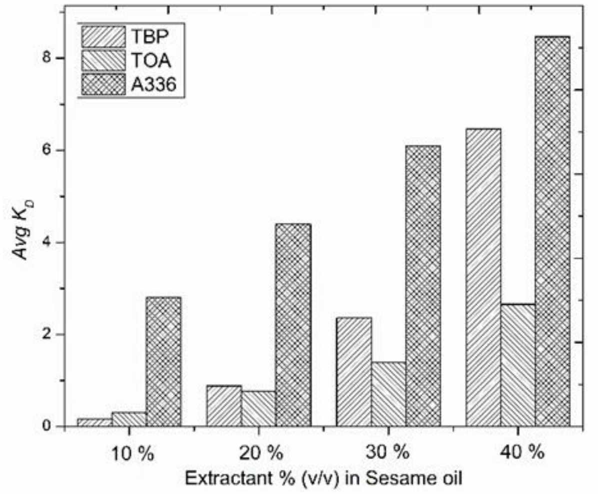

Figure 8: Variation of average distribution coefficient (Avg K_D) for different extractant in sesame oil

Effect of initial acid concentration

The higher the initial acid concentration, the higher is the amount of acid distributed in the organic phase due to the fact that higher mass transfer is caused by higher concentration gradient. Keeping the extractant concentration of TBP at 40 % v/v in sesame oil threefold increase from 3.42 to 11.5 in distribution coefficient was obtained with increase in initial acid concentration from 0.01 to 0,05 kmol.m^{-3}. Similar increasing results from 3.40 to 13.43 were obtained in rice bran oil. Five times increase in K_D (3 to 15.66) was obtained when initial acid concentration increased from 0.01 to 0.05 kmol.m^{-3} and when TBP in 40% v/v was used in sunflower oil. Increase in initial acid concentration from 0.01 to 0.05 kmol.m^{-1} at constant TOA concentration of 40 % v/v in sesame oil the distribution coefficient was from 2.04 to 3.54. Using 40 % TOA in sunflower oil at the initial acid concentration of 0.05 kmol.m^{-1}, the distribution coefficient varied from 0.81 to 2.33 and in rice bran oil the distribution coefficient varied from 0.53 to 2.44. When initial acid concentration varied between 0.01 to 0.05 kmol.m-3 and Aliquat 336 was used at 40% v/v in sesame oil, sunflower oil and rice bran oil the distribution coefficient varied in in the range of 6.51-10.11, 3 -7.89 and 5.89-10.71, respectively.

Complex Formation

With Aliquat 336 in sesame oil the 1:1 stoichiometry of the complex is supposed to exist whereas for TOA in sesame oil, the formation of 1:1 and 1:2 complexes take place. In the case of TBP in sesame oil, the formation of 1:2 and 1:3 complexes are both thought to be occurring as the solvation number is between 2 and 3. With sunflower oil, the use of TBP at lower initial acid concentration of 0.01 kmol.m^{-3} leads to the formation of 1:1 acid: extractant complexes whereas at initial acid concentration greater than 0.01 kmol.m-1 the formation of 1:2 and 1:1 complex is proposed. The case is quite the opposite with TOA in sunflower oil where there is formation of 1:2 complexes at lower initial acid concentration and 1:1 complex at high initial acid concentration. With Aliquat 336 formation of only 1:1 acid extractant complex is supposed to exist as the solvation number is less than 1. In the rice bran oil with TBP the formation of both 1:1 and 1:2 acid extractant complex is proposed as the solvation number is between 1 and 2. At low initial acid concentration of 0.01 kmol.m^{-3} with TOA in rice bran oil, the formation of 1:1 complex takes place whereas at initial acid concentration greater than 0.01 kmol.m-1, 1:2 acid: extractant complexes exist. With Aliquat 336 the value of solvation number is very less than 1 therefore 1:1 acid extractant complex formation is suggested.

The equilibrium complexation constants were obtained in the range of 1.214 – 3.397 when TBP was used in sesame oil. Higher complexation constants were obtained in the range of 1.922-7.894 and 2.192 – 6.529 when TBP was used in the sunflower oil and rice bran oil, respectively. Using TOA in sesame oil, sunflower oil and rice bran oil, equilibrium complexation constant was obtained in the range of 2.081-3.409, 0.87-2.443, 0.557-2.643, respectively. With Aliquat 336, the highest equilibrium complexation constants were obtained in sesame oil in the range of 6.622-10.621, followed by rice bran oil (K_E= 6.378-10.67) and sunflower oil (3.815- 8.390). The formation of complexes is shown in Figures 9-11.

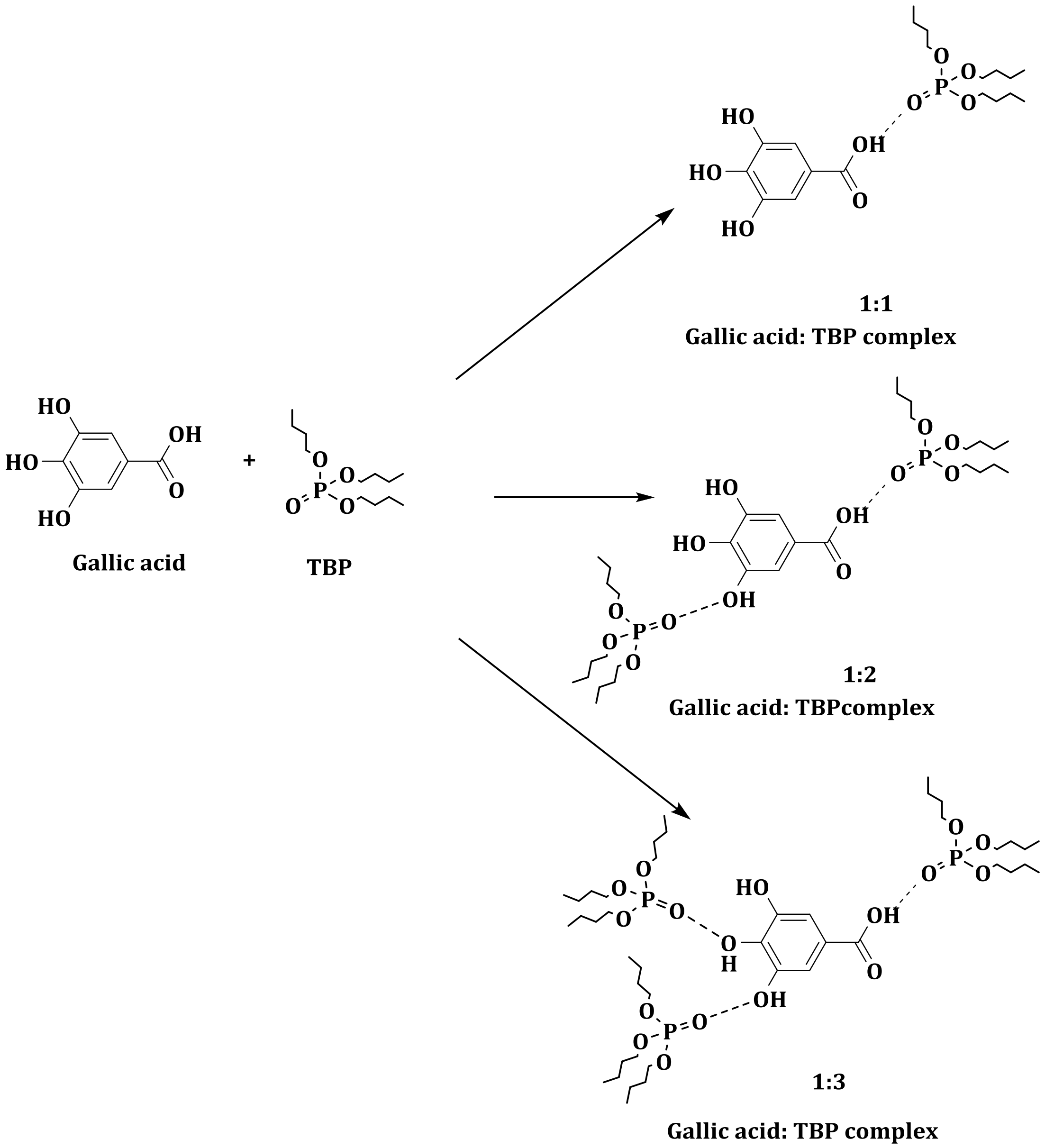

Figure 9: Gallic acid undergoing 1:1,1:2 ,1:3 complex formation with TBP

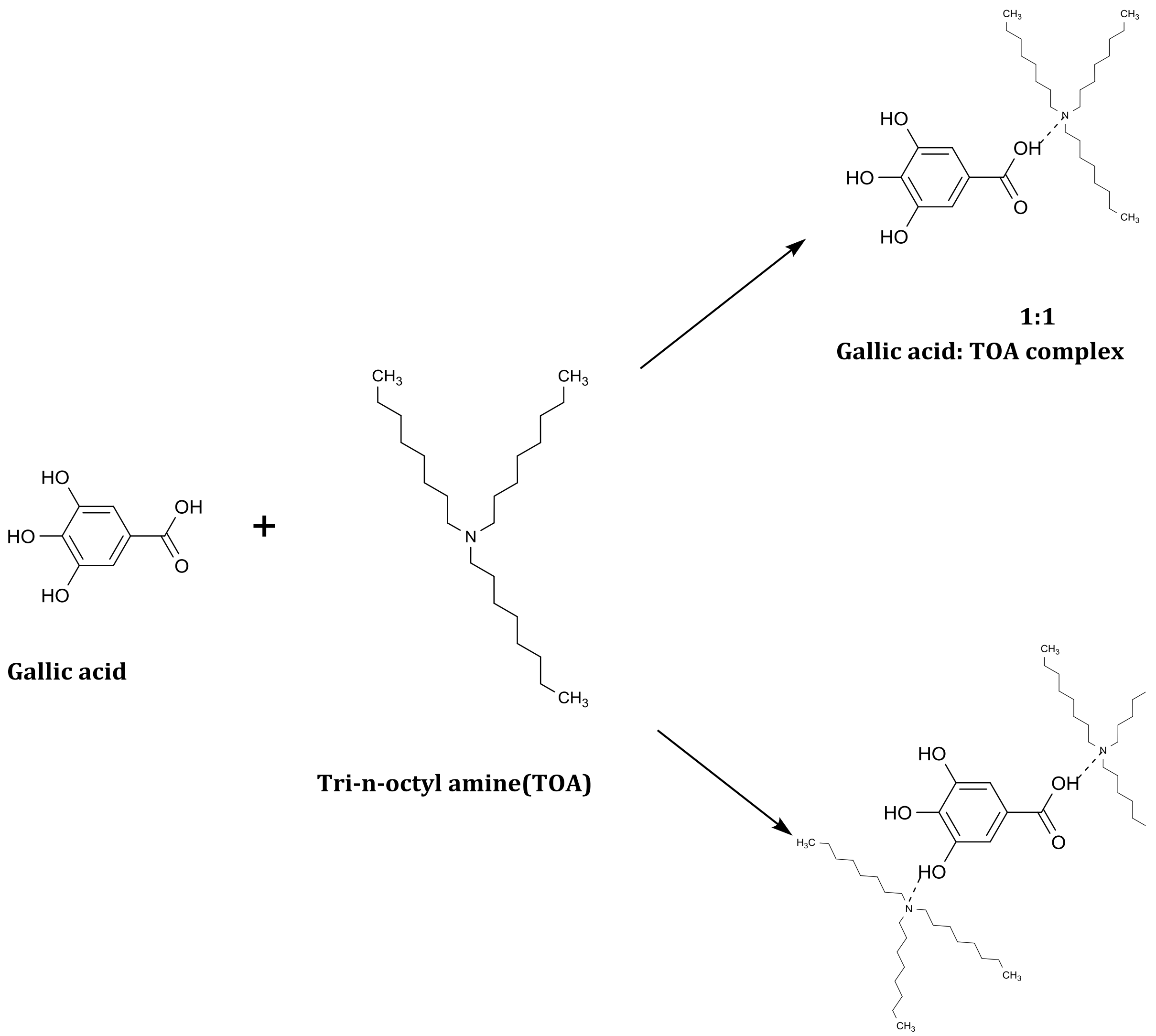

Figure 10: Gallic acid undergoing 1:1, 1:2 complex formation with TOA

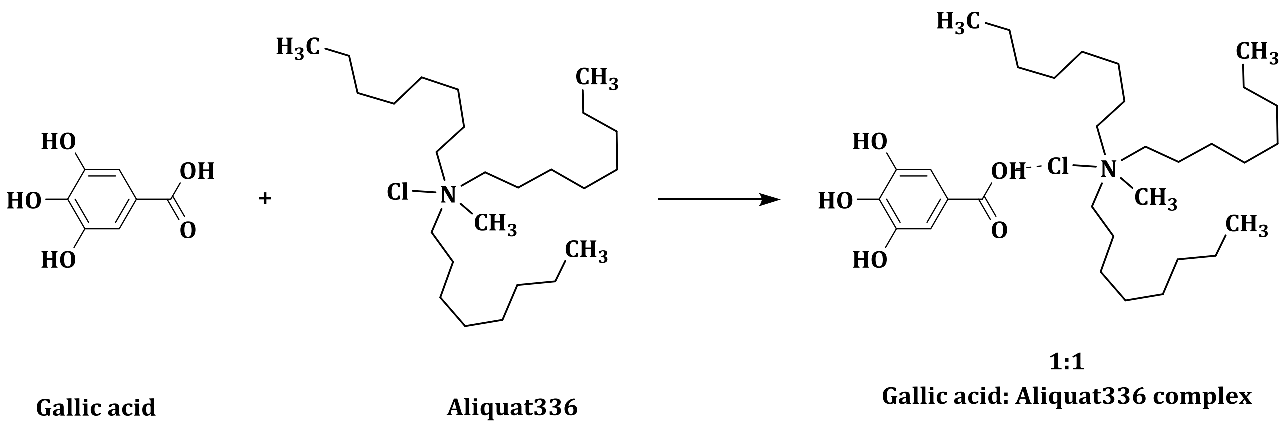

Figure 11: Gallic acid undergoing 1:1 complex formation with Aliquat336

Loading Ratio

The loading ratio values calculated using Equation (6) were 0.005-0.090(TBP-sunflower oil), 0.004-0.065(TOA-sunflower oil), 0.008-0187 (Aliquat336-sunflower oil), 0.005-0.078 (TBP-rice bran oil), 0.003-0.038 (TOA-rice bran oil), 0.009-0.206 (Aliquat336 – rice bran oil), 0.005-

0.026(TBP-sesame oil) 0.007-0.078(TOA-sesame oil), and 0.009-0.181 (Aliquat336-sesame oil). At low extractant concentration, higher loading ratio was observed when compared with loading at high extractant concentration. Further, it was observed that with the increase in acid concentrations for a constant extractant concentration, loading ratio increased for all the various combinations of solvents used in the study.

Conclusion

Non-toxic natural diluents such as sunflower oil, rice bran oil and sesame oil with different extractants such as tributyl phosphate, tri octyl amine and Aliquat 336 acid has been utilized for the physical and reactive extraction process of gallic acid which may be helpful in development of clean process for recovery of gallic acid through bio routes using reactive extraction.

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Authors' contributions

All authors contributed toward data analysis, drafting and revising the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

We have no conflicts of interest to disclose.

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