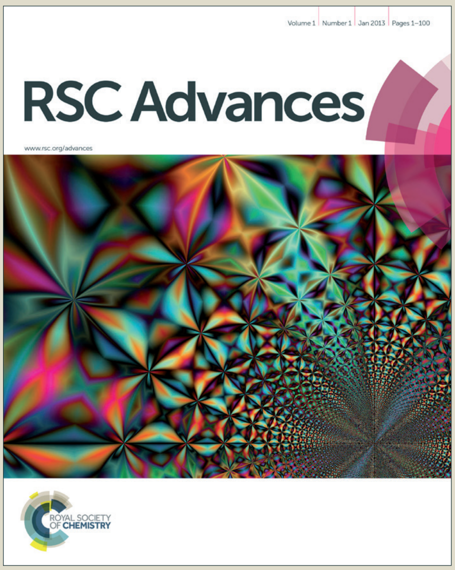
RSC Advances



Curcumin-derived green plasticizers for Poly(vinyl) chloride

José A. Saltos,[a] Wei Shi,[a] Andrew Mancuso,[a] Chong Sun,[a] Tai Park[a], Nechama Averick,[a] Kamia Punia, [a] Jimmie Fata[b] and Krishnwaswami Raja*[a]

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The efficient synthesis of Curcumin-diesters, which serve as green plasticizers for Poly (vinyl) chloride (PVC), is reported. The plasticizers lower the glass transition temperature of PVC, their cytotoxicity and leaching-resistance properties are significantly better than commercially used phthalate plasticizers which have several limitations.

1. Introduction

Poly(vinyl chloride), PVC, is today one of the most important plastic materials available in terms of consumption. It is widely used for food packaging, pharmaceutical storage, and in the medical field for a large array of medical devices[2]. PVC is relatively rigid and brittle polymer, with low thermal stability that needs to be plasticized to facilitate its processing into specific end-products[3,4].

PVC, as many of the other polymeric materials currently being used, contains plasticizers, that increase the structural flexibility, softness, workability and pliability of such polymers[5,6] by lowering their glass transition temperature. Most monomeric plasticizers are esters derived from phthalic acid, where di(2-ethylhexyl) phthalate, DEHP, and dibutyl phthalate, DBP, are the most widely used plasticizers for PVC formulations since the 1930's[3].

The low-molecular-weight phthalates are not chemically bound to the PVC matrix and can migrate into the surrounding environment, particularly after disposal[7], and studies have shown that they can be found in the soil, indoor air, and in organisms in different ecosystems[8]. Exposure of PVC-containing products to biological fluids, especially in medical applications and in the toy industry, can accelerate the migration of these additives, and considering the other possible routes of exposure, it has been estimated that the average ingestion rate of plasticizers is in the order of 8 mg/person/day[9]. One of the problems that phthalates such as DEHP and DBP present is that once they are in the environment, biodegradation is delayed due to the fact that they adsorb strongly to organic matter in deep soil and in aquatic environments[7]. Furthermore, there are studies that have reported the negative effects of phthalates on different organisms. For example, studies on rodents have shown that phthalate esters have endocrine disrupting properties that mimic or block hormones and disrupt a body's normal functions; while some plasticizers posses estrogenic features causing adverse reproductive effects on female subjects[10,11].

There have been some reports of natural plasticizers which have been used in biodegradable films[12-16]. There is however, an urgent unmet need to develop a new generation of plasticizers with improved properties such as (a) high compatibility with polymers, producing final blended products with desired thermal and mechanical properties (b) resistance to leaching (c) low- or no-toxicity to humans and the environment. Curcumin [(1*E*, 6*E*)-1, 7-bis (4-hydroxy-3-methoxyphenyl) hepta-1,6-diene 3,5-dione] is an attractive starting material for "green plasticizers" because it is Generally Regarded as Safe by the FDA (GRAS), and it is a commercially available renewable resource readily isolated from turmeric [the dried rhizomes of *Curcuma longa*]. *Curcuma Longa* is commercially grown in Hawaii, Puerto Rico and South East Asia, where over 1,100,000 tons of turmeric is produced annually[17]. Curcumin has been shown to exhibit antioxidant, anti-inflammatory[18-20], antimicrobial, and anti-carcinogenic[21-24] activities.

We hypothesized that curcumin diesters will be highly effective green plasticizers from both a synthetic perspective and with respect to properties of the end- products. The rationale behind designing curcumin-diesters lies in the fact that curcumin would serve as a rigid aromatic core attached to two flexible alkyl chains (Scheme 1); this is very similar in design to commercial phthalate plasticizers, which consist of a rigid phthalate component covalently linked to two flexible alkyl chains.

2. Experiments and Results

2.1 Synthesis of plasticizers

In a 50 mL round bottom flask, curcumin (1.36 mmole, 500 mg), stearic acid (2.2 mmole, 851.6 mg), and acetic anhydride (1.36 mmole, 1.5 mL) were added and swirled, followed by addition of DMAP (2 %mmole, 6.63 mg) at room temperature. The flask was allowed to heat to 50^{o}C in oil bath. After 24 hours, 29 µL of Millipore water was added to the solution and allowed to stir for one hour at 90^{o}C and under high vacuum. After the reaction was stopped, the reaction mixture was dissolved in methylene chloride and washed with $NaHCO_3$ (pH 8.3), and once with brine and then dried with sodium sulfate followed by filtration and solvent evaporation. The product was then purified via silica gel column chromatography using 92:8 CH_2Cl_2:EtoAc as eluent. The formation of the C18-distearate was confirmed via ^{1}H NMR by the ratio of 3:2:3 of the protons arising from the terminal CH_3 of the stearic acid component at 0.87-0.89ppm: $COCH_2R$ protons at 2.57-2.59ppm: OCH_3 protons at 3.87ppm, and by ESI-MS m/z = 901.6.

The structure of curcumin-distearate was further confirmed using and Infrared spectroscopy (IR) (see supporting information). This

[a] College of Staten Island, Department of Chemistry, 2800 Victory Blvd, Staten Island, N.Y. 10314. Telf. (718) 982-4091; Email: Krishnaswami.Raja@csi.cuny.edu
[b] College of Staten Island, Department of Biology, 2800 Victory Blvd. Staten Island, NY 10314

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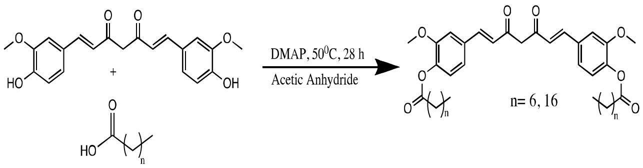
reaction is a high atom-economy esterification method, which proves to be an improvement from the catalytic dehydrative condensation reactions that are typically used.



Scheme 1. Esterification of curcumin diesters

An added advantage of our synthetic design is that stearic acid, which is produced by the hydrolysis of vegetable and animal fats, is a renewable resource. Curcumin diesters can also be synthesized via the standard acid chloride route. We also synthesized and characterized curcumin di-octanoate (CuC8) (Supporting Information). CuC18 was employed for further studies due to the fact that both starting materials, curcumin and stearic acid, are green and renewable.

2.2 Thermal Analysis

2.2.1 Plasticization procedure

Poly(vinyl)chloride (PVC) was plasticized using the solution casting method in which the polymer was dissolved in THF with varied compositions of plasticizer (weight ratios) that covers the the typical range employed commercially. The compositions of plasticizers were: 5, 15, 25, and 35% of total sample weight. (Please see supporting information for detailed procedure).

2.2.2 Thermogravimetric Analysis

Thermogravimetric (TGA) characterization was performed by heating PVC samples from room temperature to 800 ºC at 10 ºC/min under nitrogen atmosphere The TGA results of DBP shows that the thermal stability decreases with increasing plasticizer concentration, seen in the graph as a shift of the curve of PVC towards the lower temperature region where the films are decomposed faster (see supporting snformation). In the case of the curcumin distearate samples (Cu18), there is an improved shift of the curve of PVC to a higher temperature range of thermal stability when compared to DBP.

2.2.3 Differencial Scanning Calorimetry Analysis

Differential Scanning Calorimetry (DSC) was performed by heating the plasticized PVC samples between 30^{o}C and 80^{o}C at 10^{o}C /min, then cooling to -70^{o}C at 10^{o}C /min, followed by a second heating step at 10 ^{o}C /min to 120 ^{o}C. Only the second heating step was considered representative in order to calculate the glass transition temperature (T_g) of materials (Figure 1).

The T_g of unplasticized PVC was 75 ^{o}C as reported in previous literature[8]. The DSC thermograph shows that curcumin distearate decreases the T_g of PVC more efficiently than DBP. Among the curcumin distearate samples, Cu18-5% performed the poorest at lowering the T_g of PVC, while Cu18-25% had improved efficiency in lowering the T_g. The data presented also shows that the lowest T_g possible was attained at Cu18-25% (w/w) of the PVC blend mixture, the Tg is lower than the DBP-25% and DBP-35%. The capacity of the curcumin derived plasticizer to lower the T_g of plasticized PVC suggests that desired performance can be achieved with potentially less amount of our plasticizer as compared to commercially available DBP. A possible reason for

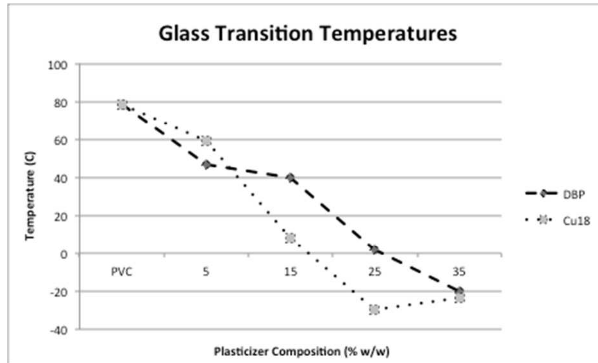


Figure 1. Glass transition temperatures (^{o}C) dependance on Curcumin distearate and DBP-plasticized PVC.

the superior performance of the Cu-18 plasticizer lies in the fact that the flexible alkyl chain component in Cu-18 (18 carbon chain) is much longer than in the case of DBP (4 carbon chain). The DSC experiment was performed multiple times to confirm the Tg values.

2.2.4 Leaching tests

The leaching behaviour or migration stability in contact with liquids of C18-plasticized PVC samples (in comparison with control DBP-plasticized PVC samples) was determined by the amount of plasticizer migrating out of the sample contacting the solvent under harsh conditions for a certain period of time. The method employed was adapted from the American Society for Testing and Materials (ASTM) Method D1239. Sample films, weighting from 23.9 to 26.3 mg as shown in table 1 (see supporting information), were immersed in dionized water or hexane. To enhance the effect of leaching, so that observations could be made in a short-time period, the temperature of the water bath was elevated to 50^{o}C for 7 days. The leaching of plasticizer was assayed spectrophotometrically using a UV Vis spectrophotometer at 280 nm for DBP-plasticized PVC, and 430 nm for curcumin distearate-plasticized PVC samples. The values reported are the average of six determinations and were calculated using the molar extinction coefficients of DBP and curcumin.

Figures 2 and 3 represent the percentage of leached plasticizer for the various compositions studied in water and hexane respectively. The leaching results presented here show that curcumin distearate is superior to DBP in both the solvents.

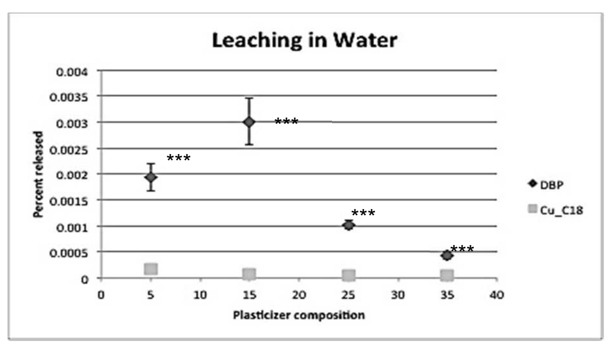


Figure 2. UV-Vis analysis results for leaching of different plasticizer compositions in Water for 7 days at 50 C. Data are presented as mean±SE (n=6 replicates. Significant differences between plasticizers at different concentrations and control are indicated as ***p<<0.0001 (Student's t-test)

It is evident from figures 2 and 3 that over the entire composition range studied, significantly less amount of curcumin distereate leaches out in comparison to DBP. The differences between DBP and Curcumin distearate can be probably attributed to the stronger intermolecular interactions between PVC and the long chains of the diester in the curcumin-derived samples in comparison the short aliphatic chains of the phthalate plasticizer.

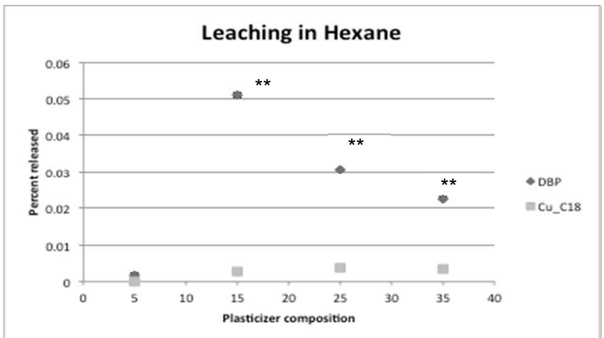


Figure 3. **UV-Vis analysis results for leaching of different plasticizer compositions in *n*-Hexane for 7 days at 50 C. Data are presented as mean±SE (n=6 replicates. Significant differences between plasticizers at different concentrations and control are indicated as ***p<<0.0001 (Student's t-test)**

2.2.5 Toxicity tests.

To test the cytotoxicity of the curcumin-based plasticizer, Hela cervix cell line was cultured in a 96-wells microplate in 100 µL medium containing 5,000 cells seeded per wells. Wells containing normal medium were used as control. After incubation for 24 hours, 10 µL of MTT was added into the wells and incubated in a humidified environment of 5% CO_2 and 37^{o}C for 2 hours. The effect of curcumin distearate on the viability of Hela cells was determined using a MTT assay, and compared to DBP. The treated cells were normalized to the control sample (media with DMSO at 0.3% v/v%). DMSO was used to solubilize the compounds and had no effect on cell viability at the concentrations used (results not shown).

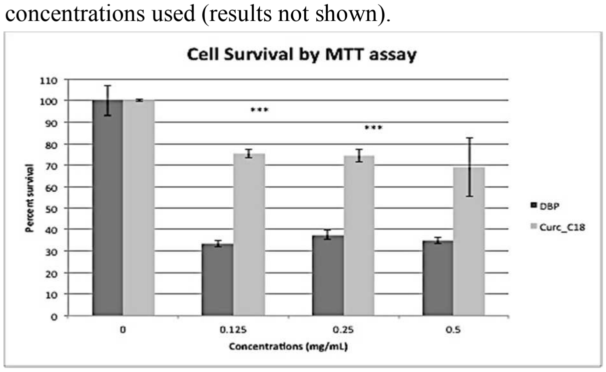


Figure 4. MTT assay results for Hela cells treated with DBP and Curc_C18 at 0 mg/ml (control) and 0.125, 0.25 and 0.50 mg/ml. Data are presented as mean±SE (n=4 replicates). Significant differences between plasticizers at different concentrations and control are indicated as ***p<<0.0001 (Student's t-test)

The three concentrations of treatment used for Hela cells showed that cell viability is above 70% for the curcumin distearate (CuC18). The cell viability was significantly different, and higher for CuC18 treated cells in comparison to DBP treated cells. DBP reduced the viability of the cells to less than 40% (33.4, 37.5 and 35.0% for each concentration respectively) Figure 4. Tickner et al (2011) reported that some plasticizers used in medical devices could impair cell proliferation, in *in vitro* studies of Sertoli cell/genocyte co-culture systems, at concentrations as low as 0.028 µg/mL.s The results presented in this preliminary cell survival assay provide a good indication of the low cytoxicity of curcumin-based plasticizers at relatively high concentrations.

2.2.6 Biodegradation Estimation

A preliminary estimate of the environmental fate of C18 was determined using the EPI Suite (BIOWIN v4.10). The probability of rapid degradation was estimated using Biowin 1 and Biowin 2 models to be 1.611 and 0.9965 respectively. Any figure higher than 0.5 is considered as likely to undergo rapid biodegradation. The MITI* Biodegradation Probability was estimated to be 1.0505 and 0.6891 using Biowin 5 and Biowin 6, respectively. Any result above 0.5 is considered to be highly likely to undergo rapid biodegradation. The above results suggest that C18 plasticizer is indeed green and environmentally friendly.

3. Conclusions

In conclusion novel green plasticizers were synthesized via a convenient esterification reaction between curcumin and carboxylic acids using minimum amount of base auxiliary (DMAP) and solvent via the mixed anhydride esterification method. The MTT assay for cell viability test using the Hela cell line indicates that the curcumin-based plasticizers have a significantly lower cytotoxicity in comparison to the DBP. In a leaching study using PVC-incorporated curcumin distearate and control DBP films, we were able to observe superior resistance to leaching in water and hexane in the former's case. Pronounced leaching in an aqueous solution observed in the case of the DBP-based films is expected to present problems related to the flexibility of polymers and may pose health and environmental risks. The reduction in glass transition observed in the case of curcumin distearate-incorporated PVC films is more pronounced than DBP plasticized films, which is an indication of the thermodynamic compatibility of the plasticizer with the PVC.

The results presented in this communication indicate the tremendous potential of curcumin diesters as plasticizers for PVC and other polymers. The mixed anhydride esterification method allows for production of the diesters in good yields and in solvent-limited conditions, making the process environmentally friendly. The C18 plasticizer is a significant improvement to DBP. Studies are currently underway to assess the effectiveness of curcumin diesters as plasticizers for a range of other polymers. Endocrine-disrupting properties of this family of plasticizers will be studied in the future.

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Appendix A. Supplementary Information

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.polymer.*XXXXXX*

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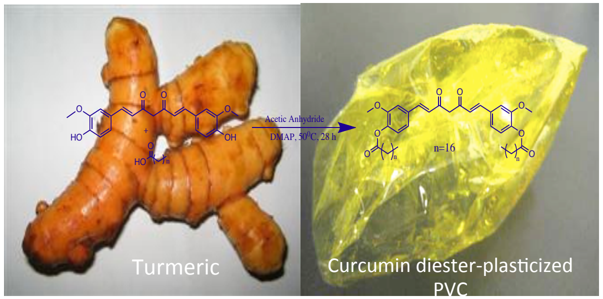
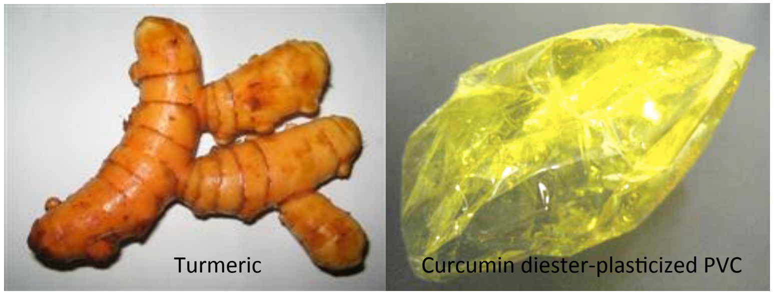
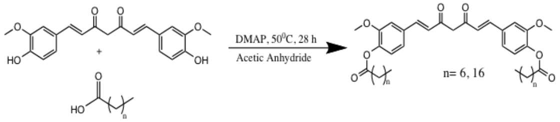


Environmentally friendly, highly effective, and non-cytoxic Curcumin-derived plasticizer for Poly(vinyl chloride) was synthesized via a high atom economic route


Turmeric
Curcumin diester-plasticized PVC


DMAP, 50⁰C, 28 h
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