LED and UV Light Utilizing Flavonoids and Limonoids to Investigate the Effect of Post-harvest Light Irradiation

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Abstract

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To investigate the effect of post-harvest light irradiation on the accumulation of flavonoids and limonoids, harvested Newhall navel oranges were continuously exposed to light-emitting diode (LED) and ultraviolet (UV) light irradiation for 6 days, and the composition and content of flavonoids and limonoids in the segments were determined using UPLC-qTOF-MS at 0, 6, and 15 days after harvest. In total, six polymethoxylated flavonoids (PMFs), five flavone *O/C*-glycosides, seven flavanone-*O*-glycosides, and three limonoids were identified in the segments. The accumulation of these components was altered by light irradiation. Red and blue light resulted in higher levels of PMFs during exposure periods. The accumulation of PMFs was also significantly induced after white light, UVB and UVC irradiation were removed. Red and UVC irradiation induced the accumulation of flavone and flavanone glycosides throughout the entire experimental period. Single light induced limonoid accumulation during exposure periods, but limonoid levels decreased significantly when irradiation was removed. Principal component analysis showed a clear correlation between PMFs and white light, between flavonoid glycosides and red light and UVC, and between limonoids and UVC. These results suggest that the accumulation of flavonoids and limonoids in citrus is regulated by light irradiation. White light, red light and UVC irradiation might be a good potential method for improving the nutrition and flavor quality of post-harvest citrus.

Keywords:

Newhall navel oranges; LED; UVs; PMFs; flavonoid glucosides; limonoids

1. Introduction

Many physical or chemical methods, such as cold storage [1], heat treatment [2], light irradiation [3], and natural antibacterial agents [4] are used to delay the loss of fruit quality after harvest. Post-harvest chemical treatments for preserving fruit are becoming less accepted by consumers due to the possible pollution or other undesired residues. Light irradiation has attracted increasing attention due to its advantages of being highly efficient and residue free as well as its ability to control decay and extend storage life. It has been reported that exposure to UVC and UVB induces large increases in resveratrol derivatives in post-harvest grapes [5]. Traditionally, UVC irradiation has been recognized as an effective sterilization method to increase the shelf-life of fruits [6,7]. Continuous white–blue light exposure increases the storage time of broccoli and contributes to a higher accumulation of antioxidant compounds [8]. These findings suggest that both ultraviolet and other lights have the potential to be applied for maintaining or enriching the health-promoting components of post-harvest fruits.

Previous studies have investigated the effect of red and blue LED light irradiation on β -cryptoxanthin accumulation in the flavedo of Satsuma mandarin (*Citrus unshiu* Marc.), the combination of ethylene and red LED light irradiation on carotenoid metabolism [9], and the regulation role of red and blue LED light irradiation in

ascorbic acid metabolism in citrus juice sacs of Satsuma mandarin (*Citrus unshiu* Marc.), Valencia orange (*C. sinensis* Osbeck), and Lisbon lemon (*C. limon* Burm.f.) [10]. To date, the effect of light irradiation on flavonoid metabolism in Newhall navel oranges or other citrus fruits has not yet been reported. Though similar studies were conducted in pear, apple, and mango, only total flavonoid content was determined, or single light irradiation was used. A global evaluation of multiple light sources regarding bioactive compounds in fruit is still lacking.

Flavonoids and limonoids are two major groups of health-promoting components in citrus fruits that exhibit a broad range of biological activities, such as antioxidant [11], anti-proliferative [12], anti-atherosclerotic [13], and neuroprotective effects [14]. Light treatment reportedly increases total flavonoid content in a wide range of species, including pear [15], apple [16], mango [6], and Brussel sprouts [17]. However, the majority of studies have only examined the impact of UVB or blue light treatment on health-promoting components in fruits, and less information is available for other lights on this subject.

Newhall navel orange (*Citrus sinensis* Osbeck) is one of the most important orange cultivars distributed worldwide for its special flavor and good performance during storage [18]. The fruits have high nutritional value, and are abundant in hesperidin, narirutin, and limonin. These compounds present health-promoting properties such as antioxidant, anticancer, anti-atherosclerotic, and vasodilatation effects [19,20]. Due to its high overall quality, Newhall navel orange has gradually become one of the leading fresh citrus cultivars in China [18,21]. Currently, China is the largest producers of Newhall navel orange in the world. By the end of 2018, the total planting area in China had reached around 2000 thousand hectares, with an annual output of approximately 4 million tons, and play an important role in citrus production in China. In this work, we investigated the effect of both UV and LED irradiation on individual flavonoids and limonoids in the edible portion (segments) of Newhall navel orange to determine the optimal light quality for maintaining or improving nutritional quality during storage.

2. Results

2.1. Identification of Flavonoids and Limonoids

From 2–16 min of retention time, every ion peak was analyzed in both low- and high-energy modes (Supplementary Materials Figures S1–S22). Each identified compound exhibited extremely similar retention times, accurate mass, and MS² ion fragments with reported information for the same compound (Table 1 and Supplementary Materials Table S1) [19]. For example, in the first observed flavonoid, the ion peak was found at 3.36 min with the quasi-molecular ion [M + H]⁺ at *m/z* 595.1659 in the low-energy mode (Figure 1a), corresponding to the reported retention time at 3.44 min with the MS ion at *m/z* 595.1652 (Table S1). Through elementary composition matching, the molecular formula was identified as C₂₇H₃₀O₁₅. In addition, in the highenergy mode, prominent MS² ion fragments were observed at *m/z* 595.16565, 325.07041, 457.11183, 379.08087, 409.09118, and 477.11946 (Figure 1b), corresponding to the reported diagnostic ions at *m/z* 595.16484 [M + H]⁺, 325.06996 [(M + H)^{-0.2}X-150]⁺, 457.11142 [(M + H)^{-0.2}X-H₂O]⁺, 379.08057 [(M + H)^{-0.2}X-96]⁺, 409.09124 [(M + H)150-2·H₂O]⁺ and 477.12020 [^{1,3}A⁺] (Supplementary Materials Table S1). Therefore, the first compound was identified as apigenin-6,8-di-C-glucoside (vicenin-2). In total, twenty-one health-promoting phytochemicals were identified in the segments of Newhall navel oranges, including six polymethoxylated flavonoids, five flavone-O/Cglycosides, seven flavanone-O-glycosides, and three limonoids (Table 1).

Figure 1. Mass chromatogram of apigenin-6,8-di-C-glucoside (Vicenin-2) in positive (a) low-energy and (b) highenergy modes.

After identification, the peak area of $[M + H]^+$ for each compound was integrally computed to acquire MS response values (Supplementary Materials Figures S23–S43), which were directly used to represent the content of each compound without further quantification.

2.2. Effect of Light Irradiation on Flavonoids and Limonoids in the Segments of Newhall Navel Oranges

Six PMFs, namely, 5,7,8,3',4'-pentamethoxyflavone (isosinensetin), 5,6,7,8,4'-pentamethoxyflavone (tangeretin), 5,7,8,4'-tetramethoxyflavone, 5,6,7,8,3',4'-hexamethoxyflavone (nobiletin), 5,6,7,3',4'-pentamethoxyflavone (sinensetin), and 3,5,6,7,8,3',4'-heptamethoxyflavone, were identified from the segments of Newhall navel oranges. The accumulation of these identified PMFs was altered in response to LED and UV light irradiation (Figure 2). In spite of light irradiation, the contents of all PMFs identified in this study significantly decreased during the irradiation period, but red and blue light maintained higher levels of PMFs compared to other lights. After removal from light irradiation, the accumulation of PMFs continued to decrease in fruit treated with red and blue irradiation, and darkness, while their content was significantly induced by white light, UVB, and UVC irradiation, with white light irradiation exhibiting the optimal effect followed by UVB (Figure 2).

Figure 2. MS response of PMFs in the segments of Newhall navel orange during light irradiation and the removal period.

Twelve flavonoid glycosides were identified in the segments of Newhall navel oranges, specifically five flavone-*O*/*C*-glycosides (apigenin-6,8-di-*C*-glucoside, diosmetin-6,8-di-*C*-glucoside, rhoifolin-4'-*O*-glucoside, chrysoeriol-6,8-di-*C*-glucoside, diosmin) and seven flavanone-*O*-glycosides (hesperidin, didymin, neoeriocitrin, narirutin, neoeriocitrin, narirutin-4'-glucoside, eriocitrin). Among these compounds, two flavone glycosides (diosmin, diosmetin 6,8-di-*C*-glucoside) and four flavanone glycosides (hesperidin, didymin, neoeriocitrin, narirutin) were increased in response to red light irradiation throughout the entire experimental period (Figure 3a). Similarly, narirutin, neoeriocitrin and didymin were also induced by UVA, UVB and UVC in spite of irradiation or removal from treatment. In the dark, the accumulation of diosmin, diosmetin 6,8-di-*C*-glucoside, hesperidin, didymin, and narirutin continued to decrease during the irradiation period, but consistently increased when the fruits were moved to natural light (Figure 3a). In contrast, the six flavonoids in Figure 3a were induced by blue light during the irradiation period, but their content rapidly decreased when removed from blue light, except for narirutin. In Figure 3b, UVA, UVB, UVC, darkness, and red light induced the accumulation of vicenin-2, rhoifolin-4'-*O*-glucoside, and narirutin-4'-glucoside throughout the entire experimental period; UVC exhibited the greatest inducing effect, and white light irradiation promoted lower levels of these compounds. However, even blue light irradiation induced the accumulation of the four flavonoids, and this effect disappeared when blue light was removed (Figure 3b). In addition, eriocitrin and neohesperidin were significantly induced by red light and UVB throughout the entire experimental period, with UVC and white light resulting in remarkable decreases in two flavonoids during the irradiation period and inducing a sharp increase when these two lights were removed. Fruit irradiated with blue light exhibited an inverse response (Figure 3c).

Figure 3. MS response of flavone and flavanone in the segments of Newhall navel orange during light irradiation and during the removal period. Flavone and flavanone (a) were mostly induced by red light, (b) UVC, and were also (c) induced by UV and darkness.

Three limonoids were identified, namely, limonin, epilimonin, and 7 α -limonoyl acetate, all of which exhibited patterns of increasing accumulation when the fruits were treated with UVA, UVB, UVC, and red and white light. In contrast, the content of limonin decreased when these forms of irradiation were removed, but the content of epilimonin in red light- and UVB-treated fruits and 7 α -limonoyl acetate in UVA- and UVB-treated fruits increased when irradiation was removed. Furthermore, the content of these three identified limonoids decreased during treatment with blue light and darkness but increased when irradiation was removed (Figure 4).

Figure 4. MS response of limonoids in the segments of Newhall navel orange during light irradiation and during the removal period.

2.3. Principal Component Analysis of Flavonoid and Limonoid Responses

Principal component analysis (PCA) was performed based on the MS response value of 21 flavonoids and limonoids identified in response to different light treatments (UVC, UVB, UVC, red, blue, white light, and darkness) and storage times (0, 6, 15 days). As shown in Figure 5, the variance explained by PC1, PC2, and PC3 was 39.8, 29.1, and 9.4%, respectively. The identified compounds were clearly distinguished according to exposure to different sources of light irradiation. Polymethoxylated flavonoids (PMFs) were clustered with white light treatment sampled at storage day 15 (WL-15 day). Flavonoid glycosides were clustered with red and UVC light treatment sampled at storage day 15 (RL-15 day, UVC-15 day), and limonoids were clustered with UVC treatment at storage day 6 (UVC-6 day). Taken together, the results of the principal component analysis are in agreement with our observations of MS response.

Figure 5. PCA model based on 21 phytochemicals in Newhall navel orange in response to different forms of light irradiation. DK: Dark; WL: White light; BL: Blue light; RL: Red light; UVA: Ultraviolet A; UVB: Ultraviolet B; UVC: Ultraviolet C; d: Days after harvest.

3. Discussion

Light irradiation has been developed as an efficient method with no pollution to extend the shelf-life and maintain the quality of vegetables and fruits. In general, visible and ultraviolet light are both intrinsic parts of sunlight, while in the natural environment, all solar UVC (<280 nm) and most UVB (280–315 nm) rays are absorbed by the stratospheric ozone layer before reaching the Earth's surface [37]. Artificial light, especially LED light that can be set to any wavelength, and is widely used in research on supplementary irradiation of various plant species. In recent years, the majority of studies on fruits treated with light irradiation have reported increases in total flavonoid content, but few studies have focused on the effect of light on individual flavonoids. For most studies, only one monochromatic light was used, while almost no studies have reported the effect of multiple light irradiation sources alone on flavonoids. In addition, all studies only investigated the effect of irradiation period. Few available studies concerned the delay effects when irradiation was removed. For citrus fruits, a relevant study reported that blue light (450 nm, 630 $\mu\text{mol}/\text{m}^2\cdot\text{s}$) induces some phenylpropanoids, such as scoparone [38], and, as reported previously, naringin and tangeretin are induced by UVC irradiation (254 nm, 0.1 W/m^2) [39]. In this study, we investigated the effect of post-harvest light irradiation on the accumulation of individual flavonoids and limonoids in the segments of Newhall navel orange simultaneously, using five kinds of LEDs and ultraviolet light within the wavelength range of 100–660 nm, as well as one type of composite white light (100 $\mu\text{mol}/\text{m}^2\cdot\text{s}$) and darkness, to identify the optimal light treatment for maintaining and improving health-promoting components in citrus fruits.

Herein, we detected 21 phytochemicals in the segments of Newhall navel oranges (*Citrus sinensis* Osbeck) and further identified six PMFs, twelve flavonoid glycosides, and three limonoids, which are almost identical to

citrus characteristic compounds reported previously [40,41,42]. Previous studies demonstrated that red and blue light treatment increases flavonoid content in microgreens [43]. Blue light, rather than red light, enhances the accumulation of flavonoids in callus cultures of *Saussurea* [44]. In the present study, we found that even red and blue light could not induce the accumulation of PMFs in the segments of Newhall navel oranges. However, red and blue light maintained higher levels of PMFs compared to other light sources during the irradiation period. More importantly, post-harvest white light (100 $\mu\text{m}^2\cdot\text{s}$) irradiation specifically enhanced the accumulation of PMFs when light was removed, suggesting that each compound responds differently to a single light source. In *Arabidopsis thaliana*, key enzymes of anthocyanin biosynthesis are strongly induced by blue light, and the induction intensity decreases in the order of UVA, UVB, and red light [45]. In this study, despite the content of twelve flavonoid glycosides all obviously increasing during blue light irradiation, these contents were dramatically decreased after removal of the light. Unlike blue light, red light significantly increased the content of diosmetin-6,8-di-C-glucoside, diosmin, hesperidin, neoeriodictyonin, narirutin, and didymelin. Furthermore, UVC remarkably elevated levels of vicenin-2, rhoifolin-4'-O-glucoside, narirutin-4'-glucoside, and stellarin-2 in fruit segments, and the accumulation effects remained after removal of the light source until the last day of observation, which was on day 15 of storage. Therefore, red light and UVC irradiation might represent a more efficient method for improving flavonoid content after harvest, which is an essential demand of post-harvest logistic systems of fresh fruits.

Compared to flavonoids, little information is available concerning the effect of light irradiation on the accumulation of limonoids. To our knowledge, the first relevant study was reported by Jairam et al. and demonstrated that limonin and nomilin were reduced in irradiated (300 gray) freeze-dried grapefruit pulp [46]. Our results show that only UVC irradiation induced limonin, epilimonin, and 7 α -limonoyl acetate during six days of continuous irradiation, despite the content decreasing again after removal of the light. Similar effects were observed in response to other light treatments. Although limonoids were first discovered as bitter citrus components, their rich biological activities were later found to promote human health, which is undoubtedly more important [40]. The above results demonstrated that both LED and UV light affect the metabolism of flavonoids and limonoids in citrus fruits.

A previous report found that irradiation of pear with UVB (280–315 nm) and/or fluorescent lamps for 10 days after harvest not only results in a significant increase in the anthocyanin, flavonoids, chlorophyll, and carotenoid concentration in the peel, but also leads to a slight increase in soluble solid and organic acid concentration and a significant increase in total sugars in the flesh [15], indicating that post-harvest irradiation can permeate through the peel of the whole fruit to affect the inner pulp. Our present study provides further evidence for this. Even so, besides the light quality, the intensity of light is another important factor affecting the accumulation of metabolites in fruits, especially in the inner part of the flesh. Therefore, when a specific intensity of light was used to irradiate fruit, the intensity of light may be different for each part of the fruit. For this point, further work is required to screen for the optional intensity of light for flesh.

Light-controlled flavonoid biosynthesis in fruits is known to be regulated by *R2R3 MYB* transcription factors. Constitutive photomorphogenic 1 (COP1) acts as a central repressor in the light signaling pathway by interacting directly with photoreceptors to mediate flavonoid biosynthesis [47]. In visible light, the function of COP1 was repressed by interacting with activated photoreceptors, and COP1 is subsequently exported from the nucleus, allowing nuclear-localized transcription factors to accumulate and induce gene expression in light-regulated processes. The expression of structural flavonoid genes is directly regulated by *R2R3 MYB* transcription factors which may be regulated by basic leucine zipper (bZIP) transcription factors such as *HY5*. Ultraviolet B radiation is strongly absorbed by tryptophan (Trp) amino acid residues in the dimeric form of a UV resistance locus 8 (UVR8) photoreceptor leading to the monomerization of UVR8. Monomeric UVR8 and COP1 form a complex that accumulates in the nucleus of the cells [48,49]. The UVR8–COP1–SPA complex stabilizes the bZIP transcription factor *HY5*, promoting the activity of different *R2R3 MYB*s for the transcription of specific flavonoid biosynthesis genes. However, the possible mechanism of limonoids in response to light irradiation needs to be further studied in the future.

4. Materials and Methods

4.1. Chemicals

The HPLC-grade acetonitrile and methanol were purchased from Sigma–Aldrich (St. Louis, MO, USA). The HPLC-grade formic acid was purchased from Fisher Scientific (Shanghai, China). Ultrapure water was acquired using the Millipore water purification System (Bedford, MA, USA).

4.2. Sample Treatment

Newhall navel oranges were collected in December 2018 from the National Citrus Germplasm Repository, Citrus Research Institute of Chinese Academy of Agricultural Sciences, Chongqing, China. Ten fruit trees with the same age and similar growth condition were marked at the same orchard before sampling. Fully ripe fruits were picked randomly from four directions on these marked trees. Then, the fruits were randomly divided into 7 groups, and 180 fruits were included in each group, and these were then treated with different wavelengths of light. For each sampling, 60 fruits were taken out, 20 fruits each as biological replicates. Treatment conditions were set using the RXZ-300D intelligent growth cabinet with adjustable light irradiation parameters, purchased from Southeast Instrument Company (Ningbo, China), and all the UV TL-D36/16 lamps were purchased from Philips Company (Netherland), and the LED RDN-500B-C lamps were purchased from Ningbo Southeast Instrument Co., Ltd. (Ningbo, China). The control group was stored at 20 °C and 90–95% relative humidity (RH) in the dark. The other groups were irradiated with UVC (10–280 nm, 100 $\mu\text{m}^2\cdot\text{s}$), UVB (270–315 nm, 100 $\mu\text{m}^2\cdot\text{s}$), UVA (315–400 nm, 100 $\mu\text{m}^2\cdot\text{s}$), blue light (470 nm, 200 $\mu\text{m}^2\cdot\text{s}$), red light (660 nm, 150 $\mu\text{m}^2\cdot\text{s}$), and white light (100 $\mu\text{m}^2\cdot\text{s}$) at 20 °C and 90–95% relative humidity (RH) for 6 days. On day 6, all lights were removed, while the same temperature and relative humidity conditions were maintained for 10 days. Fruits from each group were sampled at 0, 6, and 15 days. Sample fruits were peeled to retain only the edible portion (segments) and were immediately frozen in liquid nitrogen, powdered, and stored at –80 °C until analysis.

4.3. Extraction of Flavonoids and Limonoids

Flavonoids and limonoids were crude extracted by methanol according to the method of Xing et al. [50] with slight modifications. Four grams of segment powder from oranges were fully mixed with 7 mL methanol. At room temperature, mixtures were placed in a 300-W ultrasonic bath for 30 min and centrifuged at 5000 rpm for 20 min. The supernatant was recovered in a 25-mL brown volumetric flask. The precipitate was again extracted twice using the same method. All 21-mL mixed supernatants were finally diluted using methanol to 25 mL in brown volumetric flasks. Methanol extracts were filtered using 13-mm syringe filters with 0.22- μm PTFE membranes before passing through the UPLC column.

4.4. UPLC-*q*TOF-MS Analysis and MS Response Quantification

Samples were analyzed using UPLC/Xevo G2-S Q-TOF MS (Waters MS Technologies, Manchester, U.K.), equipped with an ACQUITY UPLC BEH C18 column (2.1 $\times$ 100 mm, 1.7 μm , UK) coupled with the UNIFI Platform in triplicate. The analysis method was performed according to Zhao et al. [51]. Briefly, the column heater was set at 40 °C and 1 μL of each sample was injected and eluted with 0.1% formic acid in water (solvent A) and acetonitrile (solvent B) with a linear gradient (0–3 min, 5–15% B; 3–8 min, 15–25% B; 8–9 min, 25–35% B; 9–13 min, 35–45% B; 13–15 min, 45–60% B; 15–16 min, 60–90% B; 16–18 min, 90–90% B; 18–20 min, 90–5% B) at a flow rate of 0.4 mL/min. Mass spectrometry was performed in the positive ionization mode in both low- (6 V) and high-energy (20–40 V) conditions. Mass spectrum conditions were as follows: source temperature of 120 °C, desolvation temperature of 400 °C, capillary voltage of 1000 V, cone voltage of 40 V, cone gas flow of 50 L/h and desolvation gas flow rate of 800 L/h. Leucine–enkephalin (m/z 556.2766) was used as the lock-mass compound at a concentration of 200 ng/mL and flow rate of 10 $\mu\text{L}/\text{min}$. Mass spectrometry data were collected between m/z 100 and 1200. The identification and quantification of target compounds were performed according to the previous methods [50,51]. The target compounds were identified by the retention time, quasi-molecular ion, molecular formula, and dominant or diagnostic MS^2 ion, and MS responses represent the relative content of the compounds.

4.5. Statistical Analysis

The peak area of the quasi-molecular ion for each identified compound was integrally computed using UNIFI software (Waters, Milford, MA, USA). Statistical differences of compound contents for each treatment point were

evaluated by Fisher's protected least squares difference (LSD) test at a 0.05 probability. Data were expressed as the mean of three biological replicates $\pm$ standard deviation (SD), using IBM SPSS Statistics software v.23 for analyses. To determine all the variables attributed to differentiate different samples, a PCA model was developed using the contents of all target compounds as the independent variables and the treatment times as the dependent variables, respectively. Three line charts and principal component analysis (PCA) were plotted by Origin Pro 2018 (Origin Lab, Northampton, MA, USA).

5. Conclusions

Twenty-one flavonoids were identified in the segments of Newhall navel oranges, specifically six PMFs, five flavone glycosides, seven flavanone glycosides, and three limonoids. The accumulation of these compounds was altered in response to exposure to different LED and UV light irradiation. The levels of PMFs decreased significantly during the irradiation period, and red and blue light maintained higher levels of PMFs. White light and UVB significantly induced the accumulation of PMFs after the fruits were removed from irradiation. Red light enhanced the content of diosmetin 6,8-di-C-glucoside, diosmin, hesperidin, neoeriocitrin, narirutin, and didymin throughout the entire experimental period with light irradiation, whereas UVC enhanced the accumulation of vicianin-2, rhoifolin-4'-O-glucoside, narirutin-4'-glucoside, and stellarin-2. Three limonoids, limonin, epilimonin, and 7 α -limonyl acetate, were induced by UVC during the irradiation period but decreased when the irradiation was removed. These results suggest that single light sources, such as red light and UVC, have great potential for improving the nutrition and flavor of citrus fruits, which can be used for post-harvest logistic systems and lighting conditions for supermarket sales. However, the specific molecular mechanisms underlying these effects require further investigation.

References

1. Chen, S. A.; Chang, E. C.; Chuang, K. R.; Chao, C. I.; Wei, P. K.; Fann, W. S., Conjugated polymer blends as emitting layer for white light led. *Abstr Pap Am Chem S* **1998**, *215*, U392-U392.
2. Goustouridis, D.; Manoli, K.; Chatzandroulis, S.; Sanopoulou, M.; Raptis, I., Characterization of polymer layers for silicon micromachined bilayer chemical sensors using white light interferometry. *Sensor Actuat B-Chem* **2005**, *111*, 549-554.
3. Chuang, C. Y.; Shih, P. I.; Chien, C. H.; Wu, F. I.; Shu, C. F., Bright-white light-emitting devices based on a single polymer exhibiting simultaneous blue, green, and red emissions. *Macromolecules* **2007**, *40*, 247-252.
4. Chitara, B.; Bhat, S. V.; Vivekchand, S. R. C.; Gomathi, A.; Rao, C. N. R., White-light sources based on composites of gan nanocrystals with conducting polymers and nanophosphors. *Solid State Commun* **2008**, *147*, 409-413.
5. Cheng, G.; Fei, T.; Zhao, Y.; Ma, Y. G.; Liu, S. Y., White phosphorescent polymer light-emitting devices based on a wide band-gap polymer derived from 3,6-carbazole and tetraphenylsilane. *Org Electron* **2010**, *11*, 498-502.
6. Amin, G.; Zaman, S.; Zainelabdin, A.; Nur, O.; Willander, M., ZnO nanorods-polymer hybrid white light emitting diode grown on a disposable paper substrate. *Phys Status Solidi-R* **2011**, *5*, 71-73.
7. Chang, C. J.; Lai, C. F.; Reddy, P. M.; Chen, Y. L.; Chiou, W. Y.; Chang, S. J., Color optimization of conjugatedpolymer/ingan hybrid white light emitting diodes by incomplete energy transfer. *J Lumin* **2015**, *160*, 145-150.

8. Chen, B.; Feng, J. C., White-light-emitting polymer composite film based on carbon dots and lanthanide complexes. *J Phys Chem C* **2015**, *119*, 7865-7872.
9. Feng, C.; Sun, J. W.; Yan, P. F.; Li, Y. X.; Liu, T. Q.; Sun, Q. Y.; Li, G. M., Color-tunable and white-light emission of one-dimensional 1-di-2-thenoyltartaric acid mixed-lanthanide coordination polymers. *Dalton T* **2015**, *44*, 4640-4647.
10. Biria, S.; Judge, K.; Malley, P.; Kahan, T.; Hosein, I., Controlling polymer morphology using white-light. *Abstr Pap Am Chem S* **2016**, 252.
11. da Silva, M. A. T.; Thomazini, E. F.; Albertini, M.; Renzi, W.; Franchello, F.; Dias, I. F. L.; Duarte, J. L.; Pocas, L. C.; Lourenco, S. A., Characterization of digital textile printing and polymer blend (pfo-dmp:P3ht) for application in manufacture of organic diodes emitting white light - woleds. *Opt Mater* **2016**, *62*, 119-131.
12. Fan, L. M.; Fan, W. L.; Li, B.; Zhao, X.; Zhang, X. T., W-shaped 1,3-di(2,4-dicarboxyphenyl)benzene based lanthanide coordination polymers with tunable white light emission. *New J Chem* **2016**, *40*, 10440-10446.
13. Chen, M. X.; Sun, R. Y.; Ye, Y. C.; Tang, H. J.; Dong, X. Y.; Yan, J. L.; Wang, K. M.; Zhou, Q.; Wang, Z. L., Application of a novel red-emitting cationic iridium(iii) coordination polymer in warm white light-emitting diodes. *Opt Mater* **2018**, *76*, 141-146.
14. Huang, J. S.; Li, G.; Wu, E.; Xu, Q. F.; Yang, Y., Achieving high-efficiency polymer white-light-emitting devices. *Adv Mater* **2006**, *18*, 114-117.
15. Kassamakov, I.; Ojala, K.; Salmia, A.; Haeggstrom, E.; Aaltonen, J.; Huber, A.; Saarikko, H.; Osterberg, M.; Oinonen, M., Characterization of dents and grooves on polymer films using scanning white light interferometry. *Optical Micro-and Nanometrology in Microsystems Technology* **2006**, 6188.
16. Kim, T. H.; Lee, H. K.; Park, O. O.; Chin, B. D.; Lee, S. H.; Kim, J. K., White-light-emitting diodes based on iridium complexes via efficient energy transfer from a conjugated polymer. *Adv Funct Mater* **2006**, *16*, 611-617.
17. Nicolai, H. T.; Hof, A. J.; Blom, P. W. M., Charge transport in white light-emitting polymers. *Organic Optoelectronics and Photonics II* **2006**, 6192.
18. Zhen, H. Y.; Xu, W.; King, W.; Chen, Q. L.; Xu, Y. H.; Jiang, J. X.; Peng, J. B.; Cao, Y., White-light emission from a single polymer with singlet and triplet chromophores on the backbone. *Macromol Rapid Comm* **2006**, *27*, 2095-2100.
19. Lee, P. I.; Hsu, S. L. C.; Lee, R. F., White-light-emitting diodes from single polymer systems based on polyfluorene copolymers end-capped with a dye. *Polymer* **2007**, *48*, 110-115.
20. Liedtke, A.; O'Neill, M.; Wertmoller, A.; Kitney, S. P.; Kelly, S. M., White-light oleds using liquid crystal polymer networks. *Chem Mater* **2008**, *20*, 3579-3586.

21. Inoue, A.; Hosokawa, T.; Haishi, M.; Ohtani, N., 4-(dicyanomethylene)-2-methyl-6-(p-dimethylaminostyryl)-4h-pyran (dcm)-doping density dependence of luminescence spectra and white emission in polymer light-emitting diodes. *Physica Status Solidi C - Current Topics in Solid State Physics, Vol 6, No 1* **2009**, 6, 334-337.
22. Jeon, S. O.; Joo, C. W.; Yook, K.; Lee, J. Y., Color control of multilayer stacked white polymer light-emitting diodes using a quantum dot as an interlayer. *Appl Phys Lett* **2009**, 94.
23. Wang, R.; Peng, J.; Qiu, F.; Yang, Y. L.; Xie, Z. Y., Simultaneous blue, green, and red emission from diblock copolymer micellar films: A new approach to white-light emission. *Chem Commun* **2009**, 6723-6725.
24. Kim, S. H.; Jin, Y.; Yu, J. Y.; Kim, J.; Song, S.; Suh, H.; Lee, K., Color stable white polymer light-emitting diodes with single emission layer. *Synthetic Met* **2010**, 160, 835-838.
25. Lee, P. I.; Hsu, S. L. C.; Lin, P. Y., White-light-emitting diodes from single polymer systems based on polyfluorene copolymers with quinoxaline derivatives. *Macromolecules* **2010**, 43, 8051-8057.
26. Madhwal, D.; Rait, S. S.; Kumar, A.; Verma, A.; Tada, K.; Onoda, M.; Bhatnagar, P. K.; Mathur, P. C., Development and characterization of an efficient bio-white polymer light-emitting diode with red and green phosphorescent dyes as dopants. *J Mater Sci* **2010**, 45, 3300-3303.
27. Hu, B.; Yao, C.; Huang, X. R., Designing of the white-light emission from a single-polymer system: Quantum theoretical study. *Polym Sci Ser a+* **2011**, 53, 1097-1105.
28. Lee, H. K.; Kim, T. H.; Park, J. H.; Kim, J. K.; Park, O. O., White-light-emitting diodes using miscible polymer blend doped with phosphorescent dye. *Org Electron* **2011**, 12, 891-896.
29. Wang, R.; Peng, J.; Qiu, F.; Yang, Y. L., Enhanced white-light emission from multiple fluorophores encapsulated in a single layer of diblock copolymer micelles. *Chem Commun* **2011**, 47, 2787-2789.
30. Jeon, Y. P.; Choo, D. C.; Kim, T. W., Color tunable white organic light-emitting devices with a hybrid 2-methyl-9,10di(2-naphthyl)anthracene small molecule/poly(2-methoxy-5-(2-ethoxyhexoxy)-1,4-phenylenevinylene) polymer emitting layer. *Thin Solid Films* **2012**, 521, 189-192.
31. Meng, L. C.; Lou, Z. D.; Yang, S. Y.; Hou, Y. B.; Teng, F.; Liu, X. J.; Li, Y. B., White organic light-emitting diodes based on a combined electrophore and monomer emission in doubly-doped polymers. *Chinese Phys B* **2012**, 21.
32. Nicolai, H. T.; Hof, A.; Blom, P. W. M., Device physics of white polymer light-emitting diodes. *Adv Funct Mater* **2012**, 22, 2040-2047.
33. Kim, J. H.; Song, W. S.; Yang, H., Color-converting bilayered composite plate of quantum-dot-polymer for high-color rendering white light-emitting diode. *Opt Lett* **2013**, 38, 2885-2888.
34. Kuo, C. P.; Chuang, C. N.; Chang, C. L.; Leung, M. K.; Lian, H. Y.; Wu, K. C. W., White-light electrofluorescence switching from electrochemically convertible yellow and blue fluorescent conjugated polymers. *J Mater Chem C* **2013**, 1, 2121-2130.

35. Liu, B. X.; Liu, Q. Q.; Tong, C. Y.; Lu, X. D.; Lu, C. L., Blue-light-emitting surface-functionalized zns nanoparticles and their transparent polymer nanocomposites with near-white light emission. *Colloid Surface A* **2013**, *434*, 213-219.
36. Leong-Hoi, A.; Claveau, R.; Flury, M.; Uhring, W.; Serio, B.; Anstötz, F.; Montgomery, P. C., Detection of defects in a transparent polymer with high resolution tomography using white light scanning interferometry and noise reduction. *Proc Spie* **2015**, 9528.
37. Nam, G. H.; Park, I. K., Cdse quantum dot-conducting polymer hybrid structure for phosphor-free white light-emitting diodes. *J Korean Phys Soc* **2015**, *66*, 785-789.
38. Kim, Y. J.; Park, B. C.; Park, J.; Kim, H. D.; Kim, N. H.; Suh, Y. D.; Kim, Y. K., White-light-emitting magnetite nanoparticle-polymer composites: Photonic reactions of magnetic multi-granule nanoclusters as photothermal agents. *Nanoscale* **2016**, *8*, 17136-17140.
39. Niu, W. Y.; Sun, J. W.; Yan, P. F.; Li, Y. X.; An, G. H.; Li, G. M., 2d l-di-toluoyl-tartaric acid lanthanide coordination polymers: Toward single-component white-light and nir luminescent materials. *Chem-Asian J* **2016**, *11*, 555-560.
40. Hrma, M.; Sichova, K.; Svoboda, J.; Vohlidal, J., Assembling of bis(tpy)fluorenes with zn²⁺ and fe²⁺ ions into metallo-supramolecular polymers with highly efficient white-light emission. *Polymer* **2017**, *122*, 22-33.
41. Lee, J. S.; Kang, B. H.; Kim, S. H.; Lee, J. W.; Lee, S. W.; Kim, S. W.; Gopalan, S. A.; Kwon, J. B.; Bae, J. H.; Kim, E. S.; Kwon, D. H.; Kang, S. W., All-solution-processed high-brightness hybrid white quantum-dot light-emitting devices utilizing polymer modified quantum dots. *Org Electron* **2017**, *42*, 393-398.
42. Li, B. N.; Liu, L.; Fu, G. R.; Zhang, Z.; Li, H. Y.; Lu, X.; Wong, W. K.; Jones, R. A., Color-tunable to direct white-light and application for white polymer light emitting diode (wpLED) of organo-eu³⁺- and organo-tb³⁺-doping polymer. *J Lumin* **2017**, *192*, 1089-1095.
43. Shen, F. Z.; He, F.; Lu, D.; Xie, Z. Q.; Xie, W. J.; Ma, Y. G.; Hu, B., Bright and colour stable white polymer lightemitting diodes. *Semicond Sci Tech* **2006**, *21*, L16-L19.
44. Niu, Y. H.; Liu, M. S.; Ka, J. W.; Bardeker, J.; Zin, M. T.; Schofield, R.; Chi, Y.; Jen, A. K. Y., Crosslinkable holetransport layer on conducting polymer for high-efficiency white polymer light-emitting diodes. *Adv Mater* **2007**, *19*, 300-309.
45. Park, J. J.; Park, T. J.; Jeon, W. S.; Kim, S. Y.; Lee, Y. K.; Jang, J.; Kwon, J. H., White polymeric light-emitting diodes based on doping of an orange ir complex in a fluorene blue polymer. *Idw '07: Proceedings of the 14th International Display Workshops, Vols 1-3* **2007**, 1033-1036.
46. Tang, K. C.; Tseng, S. R.; Li, W. S.; Meng, H. F.; Horng, S. F.; Hsu, C. S., Broad band and white phosphorescent polymer light-emitting diodes in multilayer structure. *Synthetic Met* **2008**, *158*, 287-291.

47. Park, J. J.; Park, T. J.; Jeon, W. S.; Kim, S. Y.; Lee, Y. K.; Jang, J.; Kwon, J. H., White polymeric light-emitting diodes based on doping of an orange ir complex in a fluorene blue polymer host. *Mol Cryst Liq Cryst* **2009**, *498*, 290-297.
48. Prakash, A.; Katiyar, M., White polymer light emitting diode using blend of fluorescent polymers. *16th International Workshop on Physics of Semiconductor Devices* **2012**, 8549.
49. Qin, L. J.; Zhu, Y. C.; Yang, H.; Ding, L.; Sun, F.; Shi, M.; Yang, S. P., White-light phosphorescence from binary coordination polymer nanoparticles. *Mater Chem Phys* **2013**, *139*, 345-349.
50. Song, H. J.; Shin, G. J.; Choi, K. H.; Lee, S.; Moon, D. K., White polymer light emitting diode materials introducing dendritic quinoxaline derivative: Synthesis, optical and electroluminescent properties. *Synthetic Met* **2014**, *190*, 1-7.
51. Sun, C.; Zhang, Y.; Sun, K.; Reckmeier, C.; Zhang, T. Q.; Zhang, X. Y.; Zhao, J.; Wu, C. F.; Yu, W. W.; Rogach, A. L., Combination of carbon dot and polymer dot phosphors for white light-emitting diodes. *Nanoscale* **2015**, *7*, 12045-12050.